SONORAN UNIVERSITY
OF HEALTH SCIENCES

Scientific Report

Project Title:
Evaluation of melanin production and antioxidant activity of OBLUE White Tomato Pine Bark Brightening Drink

Project Background:
Melanin is the pigment responsible for skin color and its overproduction can lead to dark spots known as hyperpigmentation. Melanin is produced by melanocytes in the skin and determines the color of skin, hair, and eyes. An increase in melanin can occur due to various factors, leading to darker patches on the skin.

OBLUE specializes in and is committed to meeting the new health needs of modern women for "good body shape, good condition, and good skin", exploring biotechnology innovations, delving into ingredient and cell rejuvenation, and providing women with natural oral nutrition solutions. Their White Tomato Pine Bark Brightening Drink is purported to reduce melanin dark spot formation leading to brighter skin appearance.

Project Objective:
The primary objective of the proposed research was to evaluate the ability of the OBLUE White Tomato Pine Bark Brightening Drink to reduce melanin production and determine the antioxidant potential using *in vitro* assays. The outcomes of this research should support the use of this product in reducing dark spot formation on the skin and provide protection from UV exposure.

Research Plan:

1. **Cell toxicity evaluation of OBLUE product**
   Cell lines: B16F10 (melanocytes)
   Assay: MTS assay (Abcam)
   Treatments: (dose range)
   - Untreated
   - White tomato & pine bark formula

   Evaluation:
   - Determination of the optimal concentration of each formula which does not lead to cellular toxicity. This determines the dose used in subsequent assays.

2. **Effect of OBLUE product on Melanin synthesis**
   Cell lines: B16F10 (melanocytes)
   Treatments: (+/- alpha-melanocyte stimulating hormone – 1 and 5 mM)
   Untreated
   White tomato & pine bark formula
   Kojik acid, 500 mM (+ control)

   Measurements:
   - Melanin intracellular content
     Cell lysate, detected by absorbance at 405 nm

Evaluation:
- Evaluate the ability of individual formulas to reduce melanin production

3. **Effect of the OBLUE product on anti-oxidant activity**

Assay: Antioxidant assay kit (Sigma Aldrich MAK334-1KT)
Treatments:
- Untreated
- White tomato & pine bark formula
- Trolox (+ control)

Measurements:
- The Antioxidant Assay Kit measures total antioxidant capacity in which Cu^{2+} is reduced by an antioxidant to Cu^{+}.

Evaluation:
- Evaluate the ability of formulas to inhibit oxidation

Project Results:

The OBLUE White Tomato Pine Bark Brightening drink has been proposed to have clinical efficacy for improved skin care including reducing dark spots and UV protection. For *in vitro* assays, the liquid product was filtered through a 0.2 mm filter for sterilization purposes.

Since *in vitro* analysis was going to be done on the OBLUE product, initial cell toxicity studies were done. B16F10 cells (melanocytes) were treated with increasing doses of the product, incubated for 24 hours, and the level of cell viability determined by standard MTS assay. As shown in Figure 1, the product demonstrated no cellular toxicity up to 20 mL product/mL cell culture media. Higher levels did result in cellular toxicity with a 50% cell cytotoxicity value of approximately 64 mL/mL. Subsequent *in vitro* assays were performed at non-toxic doses (5-20 mL/mL) in order to evaluate the biological activity of the product.

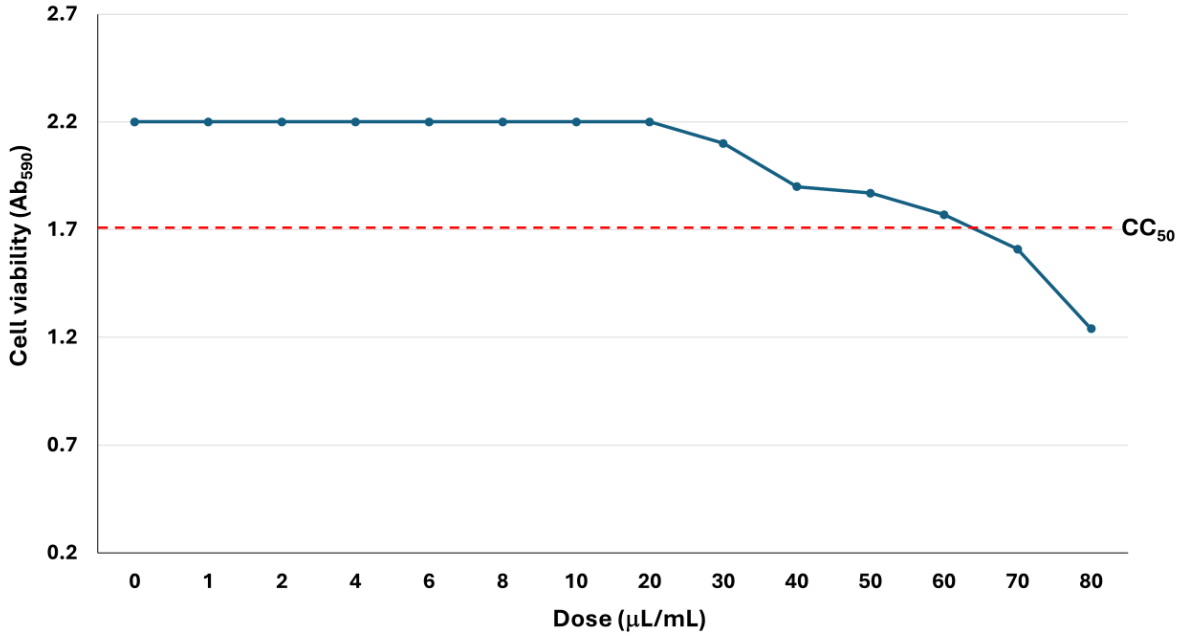

Figure 1: Cell cytotoxicity assay of OBLUE product in B16F10 cells.

Melanocytes are cells in the skin that produce melanin, the pigment that gives skin its color. Under normal conditions, melanin is made and distributed evenly to surrounding skin cells. When the process of melanin production increases or becomes uneven, dark spots (hyperpigmentation) are formed. Melanin functions to absorb and scatter UV radiation to protect skin cells' DNA. When the skin is exposed to sunlight, melanocytes get stimulated to produce more melanin as a defense. When melanocytes are overstimulated (by sun exposure, inflammation, hormones, or injury), they can produce too much melanin in certain areas, leading to localized dark patches.

The OBLUE product has been proposed to have therapeutic value by reducing dark spot formation. To test for this *in vitro*, B16F10 cells (melanocytes) were treated with a-melanin stimulating hormone (aMSH) at 1 and 5 mM concentrations to induce melanin synthesis. The cells were also treated with the OBLUE product (at 20 mL/mL) or Kojic acid (at 500 mM). After 48 hours, the cells were harvested and the level of intracellular melanin measured. Kojic acid was used as a positive control and is a natural metabolite produced by fungi and has the ability to inhibit tyrosinase activity involved in the synthesis of melanin. Kojic acid is often used as a skin-lightening agent in skin creams and lotions. When aMSH exposed melanocytes were left

untreated, the presence of intracellular melanin was observed by light microscopy (Figure 2A, red arrows). When similar cells were treated with the OBLUE product or Kojic acid, a visual reduction in intracellular melanin was observed (Figure 2A).

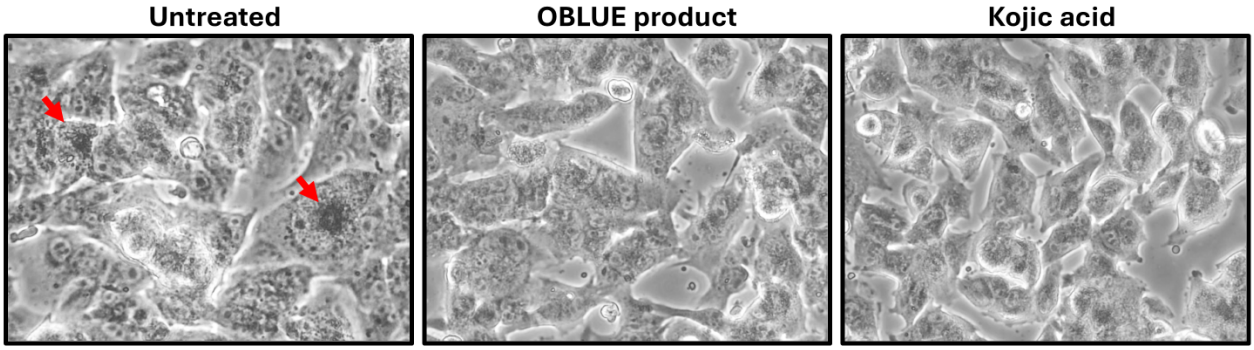

Figure 2A: Regulation of melanin production in B16F10 cells (melanocytes)

Based on this, the relative amount of melanin production was quantified. As shown in Figure 2B, treatment of the B16F10 cells with increasing doses of aMSH led to a dose dependent increase in melanin production. When the aMSH treated cells were also treated with the OBLUE product or Kojic acid, a reduction in melanin production was observed. This reduction was significant with p-values <0.05 relative to the aMSH alone treated samples (indicated by *). Both the OBLUE product and Kojic acid reduced the production of melanin similarly and to levels similar to that of the untreated cells. Treatment with the OBLUE product reduced melanin production by **82% and 77%** when treated with 1 mM or 5 mM aMSH, respectively. These results suggest that the OBLUE product was capable of inhibiting melanin production in melanocytes using an *in vitro* assay.

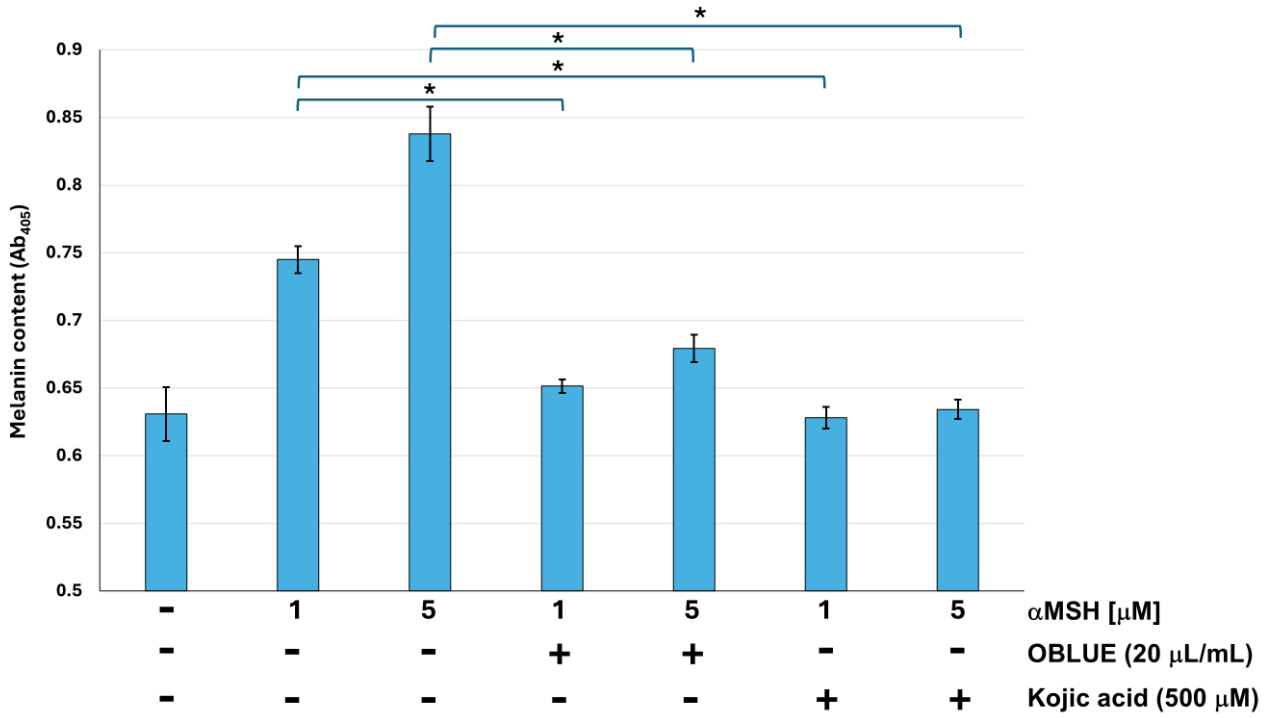

Figure 2B: Regulation of melanin production in B16F10 cells (melanocytes)

Antioxidants are known to reduce UV-induced oxidative stress. Antioxidants contribute to UV protection by neutralizing the free radicals generated after UV exposure, thereby reducing DNA damage, inflammation, and skin aging.

To test the antioxidant potential of the OBLUE product an *in vitro* assay was performed. In this assay, the antioxidant activity was measured in which Cu^{2+} is reduced by an antioxidant to Cu^{+}. The resulting Cu^{+} specifically forms a complex with a dye reagent which can be quantified. As a control, the potent antioxidant, Trolox (an analog of Vitamin E), was assessed for comparative analysis. As shown in Figure 3, Trolox demonstrated a dose-dependent increase in antioxidant activity. When the OBLUE product was tested, a similar dose-dependent increase in antioxidant activity was observed. The antioxidant activity of Trolox is often reported in the concentration range of **10 µM to 100 µM**. Since the active compounds in the OBLUE product are not known, an exact value of the mM activity cannot be assessed. However, in the assays shown, 5 mL/mL OBLUE is equivalent to 1000 mM Trolox. When the OBLUE product was dried, it was determined to contain approximately 100 mg solids/mL. The average molecular weight of plant compounds is typically between 400-800 g/mol. Based on these estimates, **10 mM Trolox is**

equivalent to approximately 6.25-12.5 mM OBLUE product. These results suggest that the OBLUE product contains potent antioxidant activity similar to that a potent standard, Trolox. In addition, Trolox has been used to reduce **oxidative stress**, including UV-induced damage. These results may suggest that the OBLUE product acts as a UV-protectant by reducing or protecting against UV-induced damage.

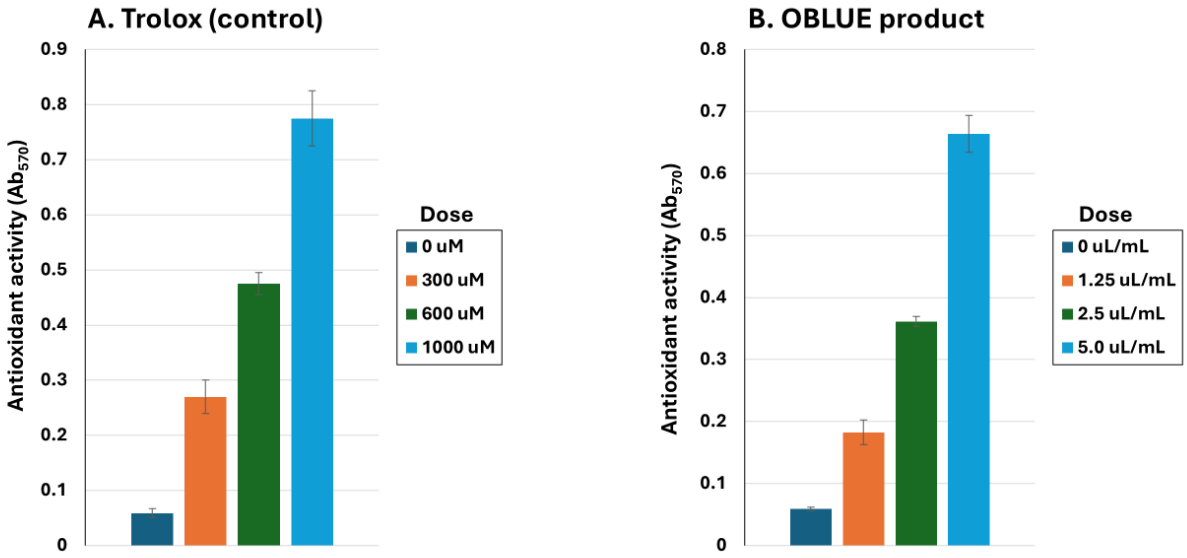

Figure 3: Antioxidant activity of OBLUE product.

Project Summary:

Independent research by our laboratory demonstrated that the OBLUE White Tomato Pine Bark Brightening drink has the ability to reduce melanin synthesis in melanocytes likely leading to the skin brightening effects observed in those who ingest the product. For these *in vitro* assays, cells were treated with a single dose of the OBLUE product at 20 mL/mL and tested for melanin production 2 days later. A typical 60kg woman would have approximately 4.2 liters of blood. The dose equivalency of 20 mL/mL *in vitro* would be approximately 85 mL in a 60kg woman. The recommended daily dose of the OBLUE product is 25 mL daily. Our *in vitro* results were performed with a single dose being administered in the presence of a melanin stimulant. Based on our results and experience, repeated daily ingestion of the OBLUE product should have cumulative effects and lead to a reduction in melanin production and the purported skin lightening effects. The potent antioxidant activity of the OBLUE product likely acts as a UV-

protectant by reducing or protecting against UV-induced cellular damage. In summary, *in vitro* scientific evaluation of the OBLUE White Tomato Pine Bark Brightening drink supports the use of this product in reducing dark spot formation and reducing UV-associated damage to the skin.

SONORAN UNIVERSITY
OF HEALTH SCIENCES

Certification:

Experimental design, procedures, and analysis were conducted at the Sonoran University of Health Sciences, Ric Scalzo Institute for Botanical Research under the guidance and supervision of Dr. Jeffrey Langland, Research Director.

Results are certified as valid based on experimental procedures performed.

March 9, 2026

Jeffrey Langland
Research Director
Sonoran University of Health Sciences
Ric Scalzo Institute for Botanical Research