## ACB Yogurt Extract Efficacy Data

<table>
<thead>
<tr>
<th>Name of Study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular Renewal Assay</strong></td>
<td><strong>ACB Yogurt Extract</strong> provided better cellular renewal than the 4.0% Glycolic Acid. <strong>ACB Yogurt Extract</strong> showed a 13.63% increase, while the synthetic showed a 18.18% increase, compared to the untreated control. <strong>ACB Yogurt Extract</strong> can be used as a suitable substitute to physical exfoliating materials.</td>
</tr>
<tr>
<td><strong>Cellular Viability Assay</strong></td>
<td><strong>ACB Yogurt Extract</strong> exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post <strong>ACB Yogurt Extract</strong> treatment. For these reasons, we can assume <strong>ACB Yogurt Extract</strong> is suitable for cosmetic applications designed to increase cell viability and metabolism.</td>
</tr>
<tr>
<td><strong>ORAC Assay</strong></td>
<td>Oxygen Radical Absorbance Capacity (ORAC) is a measure of a materials potential to protect against oxidative stress or reactive oxygen species (ROS). <strong>ACB Yogurt Extract</strong> demonstrated significant antioxidant activity by reducing the presence of ROS compared with Trolox, the vitamin E analog.</td>
</tr>
</tbody>
</table>
Abstract

ACB Yogurt Extract was evaluated for its ability to accelerate cell renewal by means of a traditional Dansyl Chloride protocol.

Methods & Materials

A 5% Dansyl Chloride was prepared by dispersing Dansyl Chloride 95% (Sigma) in petrolatum. Approximately 0.2 g of the ointment was applied to three 2cm x 2cm locations on the volar forearm of 12 (M/F) subjects between the ages of 20 and 45. The material was allowed to remain in place for 24 hours at which time any excess ointment was removed.

Two products were tested, with the remaining untreated site serving as the biological control. The products were applied in a randomized fashion. Approximately 50 μl of product was applied to the appropriate test site once per day. The sites were then examined daily under ultraviolet light (SL-3660 Long Wave Ultra Violet, Black Light Eastern Corp., Westbury, Long Island, NY) for fluorescence. The test was continued until no fluorescence was detectable at any site. The values listed reflect the average time for each product.

Results:

<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration</th>
<th>Days</th>
<th>% Cell Renewal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolic Acid</td>
<td>4%</td>
<td>18</td>
<td>18.18%</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>N/A</td>
<td>22</td>
<td>0%</td>
</tr>
<tr>
<td>Unloaded Vehicle</td>
<td>N/A</td>
<td>20</td>
<td>9.09%</td>
</tr>
<tr>
<td>ACB Yogurt Extract</td>
<td>10%</td>
<td>19</td>
<td>13.63%</td>
</tr>
</tbody>
</table>
Discussed

The results indicate that ACB Yogurt Extract is capable of increasing cellular renewal by 13.63% when compared to the untreated biological control. One of the main products produced by LAB is lactic acid. Lactic acid, being a member of the alpha hydroxy acids or AHAs, works on the skin’s lipids by dismantling them, which allow the dead cells’ outer layer to fall off (Chatelaine 1993 Sep;66(9):22). This process is done by their ability to weaken bonds which hold dead skin cells together, thus provoking the dead cells to shed from the skin’s surface. AHAs have obtained the approval of the FDA as agents capable of penetrating the skin’s barrier, thus increasing the turnover rate of cells and lowers the outer skin’s thickness. Lactic acid is an excellent moisturizing ingredient beneficial for most hyper-proliferate skin conditions.
Cellular Viability Assay Analysis

Tradename: ACB Yogurt Extract

Code: 20070

CAS #: 7732-18-5 & N/A

Test Request Form #: 372

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092
Study Director: Erica Segura
Principle Investigator: Meghan Darley

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease is ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of ACB Yogurt Extract to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.
Materials

A. Kit: PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B. Incubation Conditions: 37°C at 5% CO₂ and 95% relative humidity (RH)
C. Equipment: Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D. Cell Line: Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers: Dulbecco’s Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS)
F. Culture Plate: Falcon flat bottom 96-well tissue culture treated plates
G. Reagents: PrestoBlue™ reagent (10X)
H. Other: Sterile disposable pipette tips

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 10-fold serial dilution was performed resulting in ACB Yogurt Extract concentrations on 1%, 0.1%, and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells.
Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated.

**ACB Yogurt Extract** exhibited positive effects on cellular metabolism.

Cellular metabolism results are expressed as a percentage of the control.

![Graph showing cellular viability results for ACB Yogurt Extract at 1%, 0.10%, and 0.01% concentrations.]

**Figure 1**: Cellular Metabolism of **ACB Yogurt Extract**-treated fibroblasts expressed in terms of percent of control.

Discussion

As shown in figure 1, **ACB Yogurt Extract** exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post **ACB Yogurt Extract** treatment. For these reasons, we can assume **ACB Yogurt Extract** is suitable for cosmetic applications designed to increase cell viability and metabolism.
Oxygen Radical Absorbance Capacity (ORAC) Assay

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are detrimental to cellular structures and functional molecules (i.e. DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample’s ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of ACB Yogurt Extract.

Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2’-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.
Oxygen Radical Absorbance Capacity (ORAC) Assay

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Materials

A. Equipment: Synergy H1 Microplate reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instruments, Winooski, VT); Pipettes
B. Buffers: 75mM Potassium Phosphate (pH 7.4); Deionized H₂O
C. Reagents: 2,2'-Azobis(2-methylpropionamide) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4mM)
D. Preparation: Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.
E. Microtitre Plates: Corning 96 Well Black Side/Clear Bottom Microplates

Methods

Solutions of ACB Yogurt Extract and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37°C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

\[
AUC = 0.5 + \frac{R_2}{R_1} + \frac{R_3}{R_1} + \frac{R_4}{R_1} + \cdots + \frac{R_n}{R_1} \rightarrow \text{Where } R \text{ is fluorescence reading}
\]

\[
\text{Net AUC} = AUC_{\text{sample}} - AUC_{\text{blank}}
\]

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample’s Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE.
Results

**ACB Yogurt Extract** began exhibiting antioxidant activity at a 2.5% concentration.

![Antioxidant capacities](image)

**Figure 1**: Antioxidant capacities

**Discussion**

As shown in figure 1, **ACB Yogurt Extract** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **ACB Yogurt Extract** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

**ACB Yogurt Extract** was designed to provide moisturization and exfoliation. With the present study we can confirm that this unique ingredient is capable of providing functional benefits and potent antioxidant benefits when added to cosmetic applications.