AC Griffonia Lysate Advanced

Efficacy Data

<table>
<thead>
<tr>
<th>Type of Study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate Skin Lifting Sensorial Assay</td>
<td>91% of the volunteers were able to perceive a significant tightening effect on the area around the eye were AC Griffonia Lysate Advanced was applied. These results showed to be higher than the results obtained when using the control by itself. According to the results, we can confirm that AC Griffonia Lysate Advanced can be used to noticeably reduce the appearance of fine lines and wrinkles in an immediate basis. This is an ideal ingredient to add to cosmetic and personal care applications where intense and immediate lifting benefits are desired.</td>
</tr>
<tr>
<td>Collagen Synthesis Assay</td>
<td>Sirius Red/Fast Green Collagen Assay was conducted to assess the changes in collagen synthesis by AC Griffonia Lysate Advanced treated <em>in vitro</em> cultured human dermal fibroblasts. AC Griffonia Lysate Advanced exhibited positive collagen synthesis activity. The increase in collagen production may lead to improvement in the dermal-epidermal junction integrity as well as an improved scaffolding matrix. AC Griffonia Lysate Advanced is suitable for cosmetic applications designed to boost collagen synthesis.</td>
</tr>
<tr>
<td>Cellular Viability Assay</td>
<td>AC Griffonia Lysate Advanced exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post AC Griffonia Lysate Advanced treatment. For these reasons, we can assume AC Griffonia Lysate Advanced is suitable for cosmetic applications designed to increase cell viability and metabolism.</td>
</tr>
<tr>
<td>ORAC Assay</td>
<td>AC Griffonia Lysate Advanced exhibited antioxidant activity comparable to 200μM Trolox®. The antioxidant capacity of AC Griffonia Lysate Advanced increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.</td>
</tr>
</tbody>
</table>
AC Griffonia Lysate Advanced
Immediate Skin Lifting Benefits

Abstract

Griffonia is an exotic shrub that is native to Africa. Its seeds have been shown to contain high concentrations of 5-hydroxytryptophan (5-HTP) and high molecular weight carbohydrates. 5-HTP is a precursor of serotonin, a neurotransmitter that is capable of minimizing the appearance of fine lines and wrinkles via muscle relaxation. Unlike other precursors, 5-HTP can only be converted into serotonin in the human body.

The purpose of this study was to determine whether oAC Griffonia Lysate Advanced is capable of minimizing the appearance of fine lines and wrinkles on a short term basis. This study was conducted with 12 volunteers who applied a commercial product containing 5% AC Griffonia Lysate Advanced on the outer eye area. All subjects were asked to evaluate the improvement in skin tightening compared to the test

Materials and Methods

The study was conducted with 12 M/F volunteers between the ages of 23 and 52 years old. Neutrogena ultra-gentle facial moisturizer was used as a control. All subjects were asked to rub 1 ml of this cream on the area around their left eye. On the area around their right eye, all volunteers rubbed 1 ml of the control with 5% AC Griffonia Lysate Advanced.

3 minutes after the application of both products, the subjects were asked to rank the improvement on the perceived tightness in a range from 0 to 10, where 0 represent no perceived improvement.

Results

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Benchmark with 5% AC Griffonia Lysate Advanced</th>
<th>Benchmark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
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<tr>
<td>5</td>
<td>4</td>
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<td>8</td>
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<td>2</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Mean Improvement</td>
<td>4.25</td>
<td>1.75</td>
</tr>
</tbody>
</table>

Table 1. Rankings of the perceived skin tightening by volunteers.
AC Griffonia Lysate Advanced
Changes in Collagen Synthesis

Code: 16634
INCI Name: Griffonia Simplicifolia Seed Extract
Suggested Use Levels: 1.0-10.0%

MTT Assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abs 1</th>
<th>Abs 2</th>
<th>Abs 3</th>
<th>Via 1</th>
<th>Via 2</th>
<th>Via 3</th>
<th>Mean</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% AC Griffonia Lysate Advanced</td>
<td>0.321</td>
<td>0.316</td>
<td>0.315</td>
<td>102</td>
<td>101</td>
<td>100</td>
<td>101</td>
<td>1</td>
</tr>
<tr>
<td>0.01% Sodium Ascorbate</td>
<td>0.317</td>
<td>0.325</td>
<td>0.307</td>
<td>101</td>
<td>104</td>
<td>98</td>
<td>101</td>
<td>3</td>
</tr>
<tr>
<td>Untreated Treatment</td>
<td>0.322</td>
<td>0.05</td>
<td>0.307</td>
<td>103</td>
<td>97</td>
<td>100</td>
<td>100</td>
<td>3</td>
</tr>
</tbody>
</table>

MTT Assay

![MTT Assay Graph]
## Procollagen Assay

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Abs 1</th>
<th>Abs 2</th>
<th>Abs 3</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>Mean</th>
<th>Stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% AC Griffonia Lysate Advanced</td>
<td>1.137</td>
<td>1.116</td>
<td>1.091</td>
<td>3050</td>
<td>2863</td>
<td>2656</td>
<td>2856</td>
<td>197</td>
</tr>
<tr>
<td>0.01% Sodium Ascorbate</td>
<td>1.072</td>
<td>1.115</td>
<td>1.067</td>
<td>2509</td>
<td>2855</td>
<td>2471</td>
<td>2611</td>
<td>211</td>
</tr>
<tr>
<td>Untreated</td>
<td>1.048</td>
<td>0.99</td>
<td>1.017</td>
<td>2334</td>
<td>1960</td>
<td>2126</td>
<td>2140</td>
<td>187</td>
</tr>
</tbody>
</table>

### Bar Chart

- **1% AC Griffonia Lysate Advanced**
- **0.01% Sodium Ascorbate**
- **Untreated**
### Ratio Procollagen Production to Viable Cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PC 1</th>
<th>PC 2</th>
<th>PC 3</th>
<th>Via 1</th>
<th>Via 2</th>
<th>Via 3</th>
<th>PC/Via 1</th>
<th>PC/Via 2</th>
<th>PC/Via 3</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% AC Griffonia Lysate Advanced</td>
<td>3050</td>
<td>2863</td>
<td>2656</td>
<td>102</td>
<td>101</td>
<td>100</td>
<td>30</td>
<td>28</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>0.01% Sodium Ascorbate</td>
<td>2509</td>
<td>2855</td>
<td>2471</td>
<td>101</td>
<td>104</td>
<td>98</td>
<td>25</td>
<td>28</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Untreated</td>
<td>2334</td>
<td>1960</td>
<td>2126</td>
<td>103</td>
<td>97</td>
<td>100</td>
<td>23</td>
<td>20</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>

#### Additional Data
- 1% AC Griffonia Lysate Advanced: 28
- 0.01% Sodium Ascorbate: 26
- Untreated: 21

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**Graph:**
![Graph showing Ratio Procollagen Production to Viable Cells](image-url)
Discussion

According to the results, 91% of the volunteers were able to perceive a significant tightening effect on the area around the eye were AC Griffonia Lysate Advanced was applied. These results showed to be higher than the results obtained when using the control by itself.

Figures 1 and 2 illustrate the changes in wrinkle characteristics on subject 3. Figure one was taken before initiation of the study and figure 2 was taken after application of the control with 5% AC Griffonia Lysate Advanced. The circled areas clearly show a reduction in the overall appearance of wrinkles.

Figures 3 and 4 illustrate the changes in wrinkle appearance on subject 8. The left circles indicate the difference in a deep wrinkle before and after application of 5% AC Griffonia Lysate Advanced. Clearly the wrinkle was significantly minimized after application. The bigger circles point out the differences in wrinkle depth and length before and after application of the tested material. A noticeable reduction in wrinkles characteristics can be perceived.

According to the results, we can confirm that AC Griffonia Lysate Advanced can be used to noticeably reduce the appearance of fine lines and wrinkles in an immediate basis. For this reason, this exceptional ingredient is ideal to add to cosmetic and personal care applications where intense and immediate lifting benefits are desired.
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 AC Griffonia Lysate Advanced.

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.
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-methylpropionamidine) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM)

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 AC Griffonia Lysate Advanced 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

AC Griffonia Lysate Advanced began exhibiting antioxidant activity at a 0.05% concentration.

Discussion

As shown in figure 1, AC Griffonia Lysate Advanced exhibited antioxidant activity comparable to 50µM Trolox®. The antioxidant capacity of AC Griffonia Lysate Advanced increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

AC Griffonia Lysate Advanced was designed to provide anti-aging, tightening, and lifting properties. With the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.
**Tradename:** AC Griffonia Lysate Advanced

**Code:** 16634

**CAS #:** 999999-99-4

**Test Request Form #:** 368

**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 AC Griffonia Lysate Advanced 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.
Cellular Viability Assay Analysis

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 AC Griffonia Lysate Advanced 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.

**AC Griffonia Lysate Advanced** at all concentrations is able to increase cellular metabolism compared to the control.

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

![Graph showing cellular metabolism results](image)

**Figure 1:** Cellular Metabolism of AC Griffonia Lysate Advanced-treated fibroblasts expressed in terms of percent of control.

Discussion

As shown in figure 1, **AC Griffonia Lysate Advanced** exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post **AC Griffonia Lysate Advanced** treatment. For these reasons, we can assume **AC Griffonia Lysate Advanced** is suitable for cosmetic applications designed to increase cell viability and metabolism.