Active Concepts
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Phyto-Biotics Perilla Efficacy Data

Code: 40600
INCI Name: Perilla Frutescens Extract
CAS #: 90082-61-4
EINECS #: 290-151-0

Type of Study	Results
Cellular Viability	Phyto-Biotics Perilla exhibited positive results by increasing cell metabolism. The increase in fluorescent signal indicates an increase in cellular metabolism and viability post Phyto-Biotics Perilla treatment.
ORAC Assay	Phyto-Biotics Perilla exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of Phyto-Biotics Perilla increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.
IL-6 Elisa Assay	Phyto-Biotics Perilla exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using Phyto-Biotics Perilla appears to be dose dependent. This decrease in IL-6 production indicates a reduced inflammatory environment which could decrease the signs of aging and reduce the formation of fine lines and wrinkles.
Moisturizing Assay	As evidenced in a 4 week efficacy study of Phyto-Biotics Perilla on skin, moisture levels were improved by 74.2% after 24 hours and by 143.6% after 4 weeks when compared to the untreated control. Comparisons of the base lotion to the Experimental Lotion containing 2.0% Phyto-Biotics Perilla demonstrate the experimental material moisturized the skin 21.0% better after 24 hours. After four weeks the base lotion containing 2.0% Phyto-Biotics Perilla moisturized skin 48.3% better than the base lotion alone.

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Cellular Viability Assay Analysis

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Tradename: Phyto-Biotics Perilla

Code: 40600

CAS #: 90082-61-4

Test Request Form #: 360

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 **Phyto-Biotics Perilla** 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

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Materials

A.	**Kit:**	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 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 **Phyto-Biotics Perilla** 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.

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Results

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

Phyto-Biotics Perilla at all concentrations is able to increase cellular metabolism compared to the control.

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

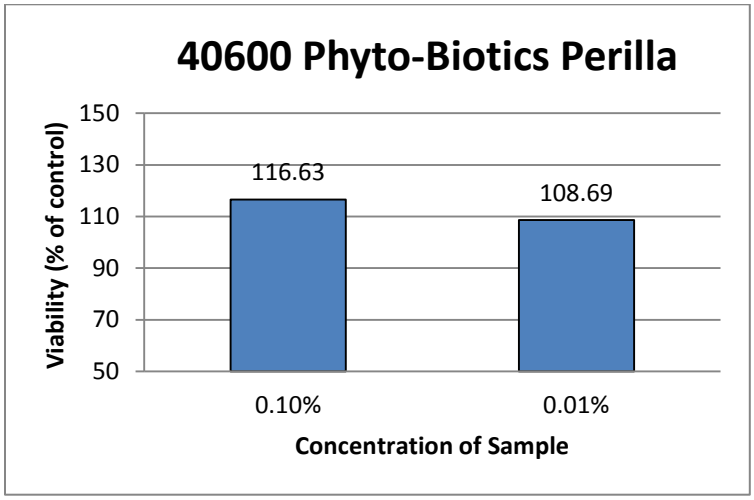

Figure 1: Cellular Metabolism of **Phyto-Biotics Perilla**-treated fibroblasts expressed in terms of percent of control.

Discussion

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

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: Phyto-Biotics Perilla

Code: 40600

CAS #: 90082-61-4

Test Request Form #: 55

Lot #: NC120814-C

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

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC)

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 **Phyto-Biotics Perilla**.

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.

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Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	75mM Potassium Phosphate (pH 7.4); Deionized H_2O
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 **Phyto-Biotics Perilla** 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 a 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:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

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.

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Results

Phyto-Biotics Perilla exhibited strong antioxidant activity at a 0.1% concentration.

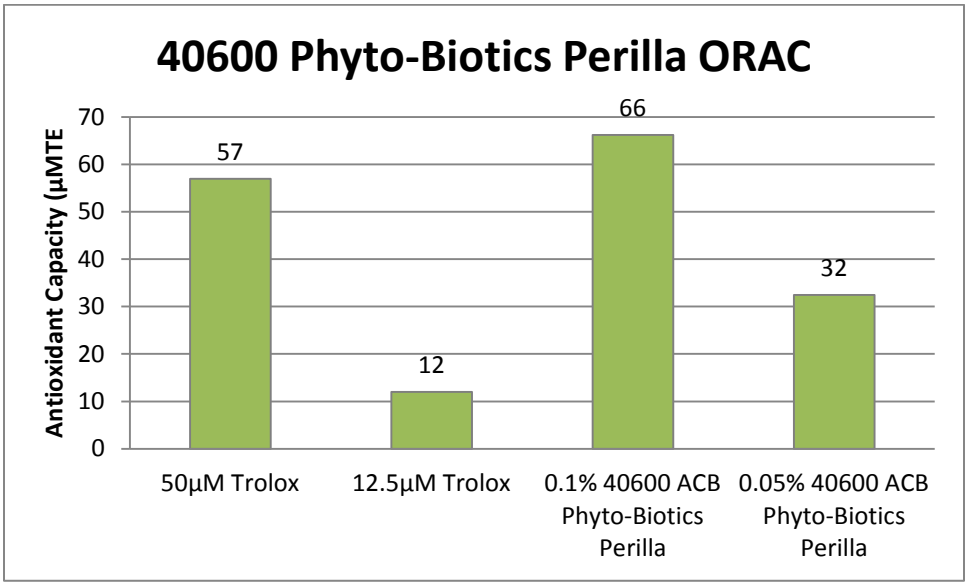

Figure 1: Antioxidant capacities

Discussion

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

IL-6 ELISA Analysis

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Tradename: Phyto-Biotics Perilla

Code: 40600

CAS #: 90082-61-4

Test Request Form #: 404

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

Test Performed:
Interleukin (IL)-6 Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

Interleukin-6 is a proinflammatory cytokine known to play an active role in inflammation, immunology, bone metabolism, reproduction, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B (NF-κB) pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP's are responsible for breaking down the extracellular matrix and collagen in the skin leading to wrinkles, fine lines, and loss of skin elasticity. Reducing the level of IL-6 and other inflammatory mediators is believed to slow down degradation of the skin matrix and, possibly, stimulate its replenishment.

Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in **Phyto-Biotics Perilla**-treated *in vitro* cultured human dermal fibroblasts.

Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IL-6. Monoclonal antibodies specific for IL-6 epitopes are coated on a microtiter plate. In positive samples, IL-6 will bind to these antibodies and are tagged a second time with another IL-6-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of collagen in each sample.

IL-6 ELISA Analysis

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Materials

A.	**Kit:**	IL-6 ELISA Kit (Biosource; KAC1261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
C.	**Equipment:**	Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; 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); Amphotericin (45pg/mL)
F.	**Culture Plate:**	Falcon flat bottom 12-well tissue culture treated plates
G.	**Reagents:**	Lipopolysaccharide (LPS) (1µg/mL)
H.	**Other:**	Sterile disposable pipette tips; wash bottles

Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, 0.01% concentrations of **Phyto-Biotics Perilla** were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 24 hours. Complete media containing 1µg/mL LPS was used as the positive controls and complete DMEM was used a negative control.

Standards were prepared in concentrations ranging from 2476pg/mL to 0pg/mL. 50µL of Solution B was added to wells for standards and assay controls and 50µL of Solution A was added to experiment wells. 100µL of standards, controls, and samples were added to appropriate wells. After a one hour incubation at room temperature and washing, 50µL Solution A and 100µL anti-IL-6 conjugate was added to all wells. Following a one hour incubation and washing, 100 µL chromagen solution was added for the colorimetric reaction. One-hundred µL stop solution was added to stop the reaction after 15 minutes. The optical density was read at 450nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-6 concentration of **Phyto-Biotics Quercus** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

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Results

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

Phyto-Biotics Perilla, at concentrations of 1%, 0.1%, and 0.01% was able to decrease IL-6 production compared to our positive control.

IL-6 levels are expressed by the following formula:

$$= Average_{IL-6\ Concentrations} \times Dilution\ Factor$$

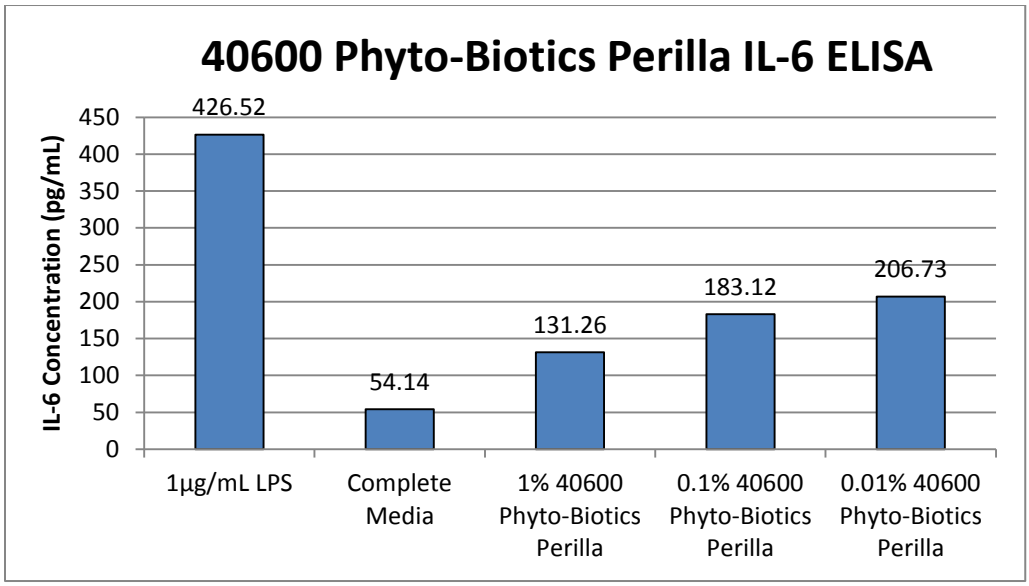

Figure 1: Phyto-Biotics Perilla-treated fibroblasts IL-6 concentrations

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IL-6 production percent decrease is calculated by the following formula:

$$= \frac{Positive\ Control_{\ Avg.Concentration} - Sample_{\ Avg.Concentration}}{Positive\ Control_{\ Avg.Concentration}} \times 100$$

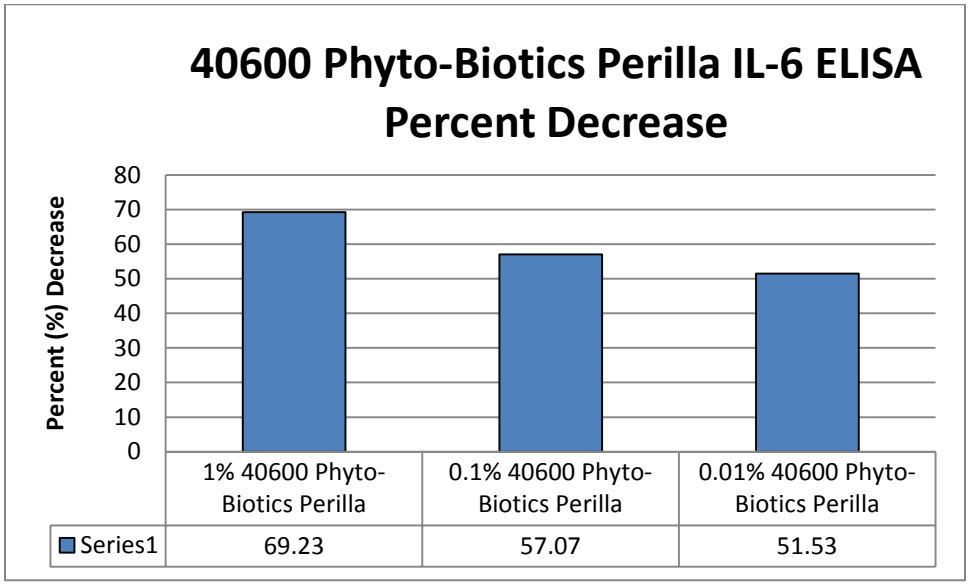

Figure 2: Percent decrease in IL-6 production compared to positive control

Discussion

As shown in figure 1, **Phyto-Biotics Perilla** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **Phyto-Biotics Perilla** appears to be dose dependent. This decrease in IL-6 production indicates a reduced inflammatory environment which could decrease the signs of aging and reduce the formation of fine lines and wrinkles. For these reasons, we can assume **Phyto-Biotics Perilla** is suitable for cosmetic applications designed to provide soothing and anti-aging properties.

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Moisturization/ Hydration Assay

Tradename: Phyto-Biotics Perilla

Code: 40600

CAS #: 90082-61-4

Test Request Form #: 495

Lot #: 27225

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

Test Performed:
Moisturization/ Hydration Assay

Introduction

An *in-vivo* study was conducted over a period of three weeks to evaluate the moisturization benefits of **Phyto-Biotics Perilla**. 10 M/F subjects between the ages of 23-45 participated in the study. Results indicate that this material is capable of significantly increasing moisturization compared to the control.

The Moisturizaiton Assay was conducted to assess the moisturizing ability of **Phyto-Biotics Perilla**.

Materials

A. Equipment: DermaLab Skin Combo (Hydration/ Moisture Pin Probe)

Methods

The moisture module provides information about the skin's hydration by measuring the conducting properties of the upper skin layers when subjected to an alternating voltage. The method is referred to as a conductance measurement and the output is presented in the unit of uSiemens (uS). A moisture pin probe is the tool used to gather hydration values.

10 volunteers M/F between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. A Dermalab Corneometer was used to measure the moisture levels on the subject's volar forearms. The Corneometer is an instrument that measures the amount of water within the skin. The presence of moisture in the skin improves conductance therefore results in higher readings than dry skin. Therefore the higher the levels of moisture, the higher the readings from the Corneometer will be. Baseline moisturization readings were taken on day one of the study.

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Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken immediately after application of test materials and then weekly for 3 weeks. The test material consisted of 2.0% **Phyto-Biotics Perilla** in a base lotion.

For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

Phyto-Biotics Perilla showed very high moisturizing capabilities at a 2.0% concentration. Please note, each value is an average of three consecutive readings per test site.

Averages	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week
2.0% Phyto-Biotics Perilla in Base Lotion	150.0	164.9	167.8	174.1	203.4	99.6	89.7
Base Lotion	124.0	136.8	126.5	126.4	137.2	72.0	70.2
Untreated	86.1	86.9	87.0	81.4	83.5	65.5	62.6

Chart 1. Average Moisture Increase and Regression Scores of Individual Test Sites

Percent (%) Change	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week
Base Lotion vs. Untreated	44.0	57.4	45.4	55.3	64.3	9.9	12.3
2.0% Phyto-Biotics Perilla + Base Lotion vs. Untreated	74.2	89.8	92.9	114.0	143.6	52.1	43.3
2.0% Phyto-Biotics Perilla in Base Lotion vs. Base Lotion	21.0	20.5	32.7	37.8	48.3	38.4	27.7

Chart 2. Comparative Moisture Increase and Regression Scores Between Individual Test Sites

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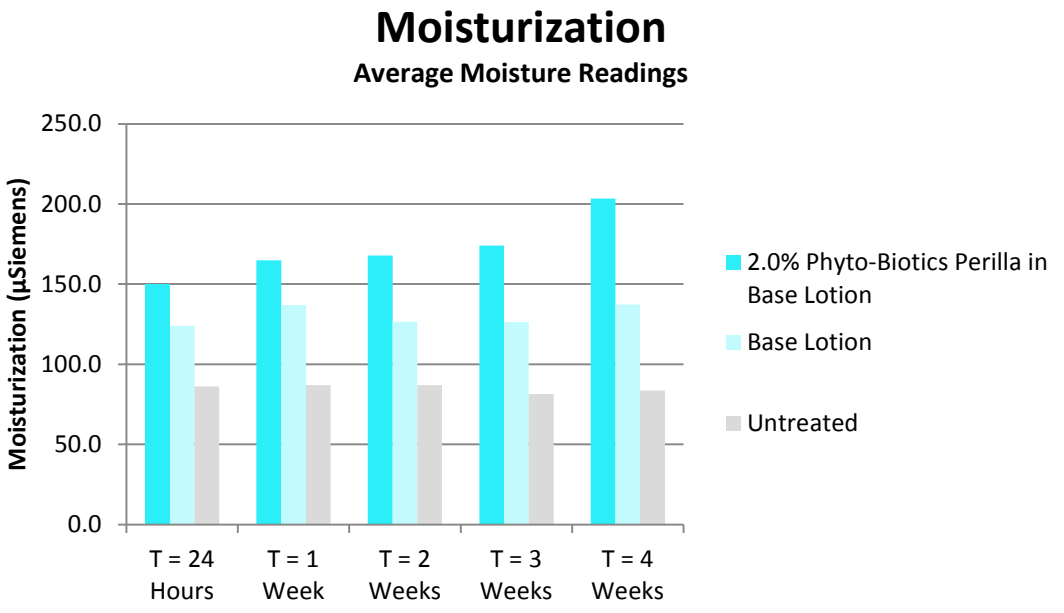

Figure 1. Average increase in moisturization per test site

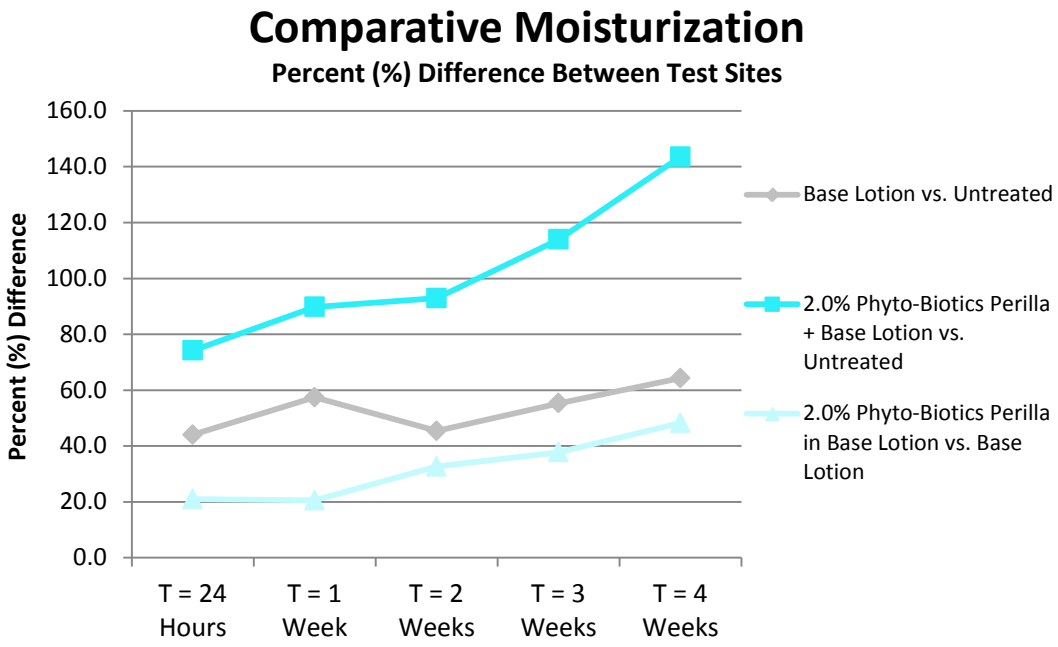

Figure 2. Percent difference in moisturization between two test sites over four weeks

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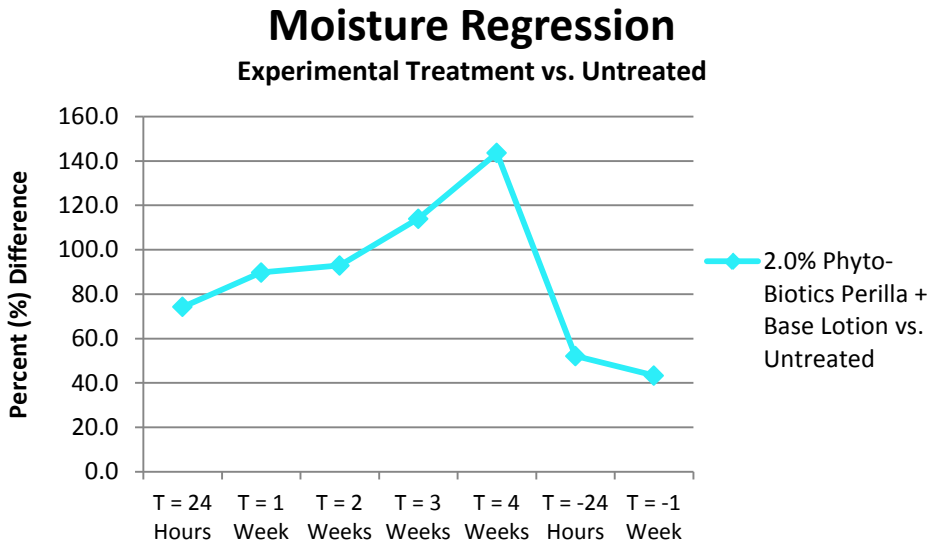

Figure 3. Regression in skin moisturization after application of experimental material ceased

Discussion

As evidenced in a 4 week efficacy study of **Phyto-Biotics Perilla** on skin, moisture levels were improved by 74.2% after 24 hours and by 143.6% after 4 weeks when compared to the untreated control. Comparisons of the base lotion to the Experimental Lotion containing 2.0% **Phyto-Biotics Perilla** demonstrate the experimental material moisturized the skin 21.0% better after 24 hours. After four weeks the base lotion containing 2.0% **Phyto-Biotics Perilla** moisturized skin 48.3% better than the base lotion alone. Results indicate that **Phyto-Biotics Perilla** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

Furthermore, when examining the moisture levels on the skin after application of test materials stopped, it was determined that **Phyto-Biotics Perilla** is capable of sustaining increased skin moisturization when compared to the skin site that remained untreated through the duration of the study. After 24 hours, the site testing 2.0% **Phyto-Biotics Perilla + Base Lotion** was approximately 52.0% more moisturized than the site which did not receive treatment. After one week, the experimental test site was still yielding moisturization results that were 43.3% higher than the untreated site. Additionally, in comparison to the site tested with the base lotion alone, the site treated with 2.0% **Phyto-Biotics Perilla + Base Lotion** moisturized the skin 38.4% better after 24 hours and was still 27.7% more effective in moisturizing the skin when reading were taken one week after the applications of both test materials ceased.

Phyto-Biotics Perilla was designed to provide moisturizating benefits, however with the present study we can confirm that this succulent botanical ingredient is not only capable of providing protective benefits but also ideal for moisturizing and skin hydrating personal care applications.