Active Concepts
Tomorrow's Vision... *Today!*®

Phyto-Biotics Acai®
Efficacy Data

Code: 16587
INCI Name: Euterpe Oleracea Fruit Extract
CAS #: 999999-99-4
EINECS #: 310-127-6

Name of Study	Type of Study	Results
ORAC Assay	*In-vitro*	**Phyto-Biotics Acai®** exhibited potent antioxidant activity comparable to 100µM Trolox®. The antioxidant capacity of **Phyto-Biotics Acai®** 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	*In-vitro*	**Phyto-Biotics Acai®** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **Phyto-Biotics Acai®** 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 Acai®** is suitable for cosmetic applications designed to provide soothing and anti-aging properties.
Cellular Viability Assay	*In-vitro*	**Phyto-Biotics Acai®** exhibited positive results by increasing cellular metabolism. This data indicates that **Phyto-Biotics Acai®** is idea for cosmetic applications designed to increase cell viability and metabolism.
Moisturization Assay	*In-vivo*	As evidenced in a 4 week efficacy study of **Phyto-Biotics Acai®** on skin, moisture levels were improved by 51.17% after 24 hours and by 102.48% after 4 weeks when compared to the untreated control. When compared to the base cream **Phyto-Biotics Acai®** improved moisturization by 12.08% and after 4 weeks **Phyto-Biotics Acai®** improved moisturization by 27.25%. Results indicate that **Phyto-Biotics Acai®** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

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Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: Phyto-Biotics Acai®

Code: 16587

CAS #: 999999-99-4

Test Request Form #: 71

Lot #: NC120806-G

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 Acai®**.

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 Acai®** 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 Acai® began exhibiting potent antioxidant activity at 0.025% concentration.

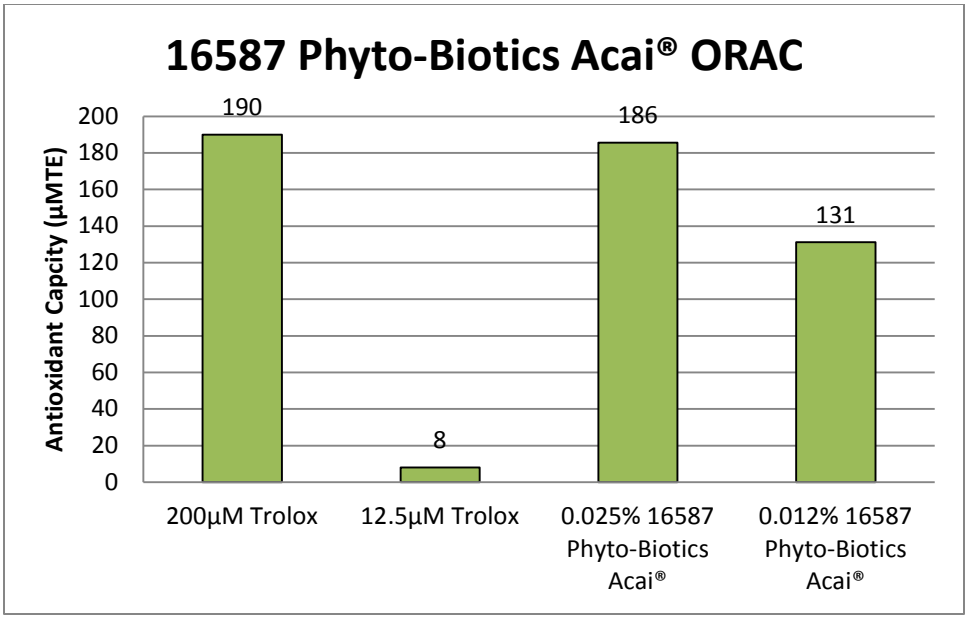

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **Phyto-Biotics Acai**® exhibited potent antioxidant activity comparable to Trolox®. The antioxidant capacity of **Phyto-Biotics Acai**® 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 Acai®

Code: 16587

CAS #: 999999-99-4

Test Request Form #: 402

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 Acai®**-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.

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IL-6 ELISA Analysis

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 Acai®** 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 Acai®** 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 Acai®, 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:

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

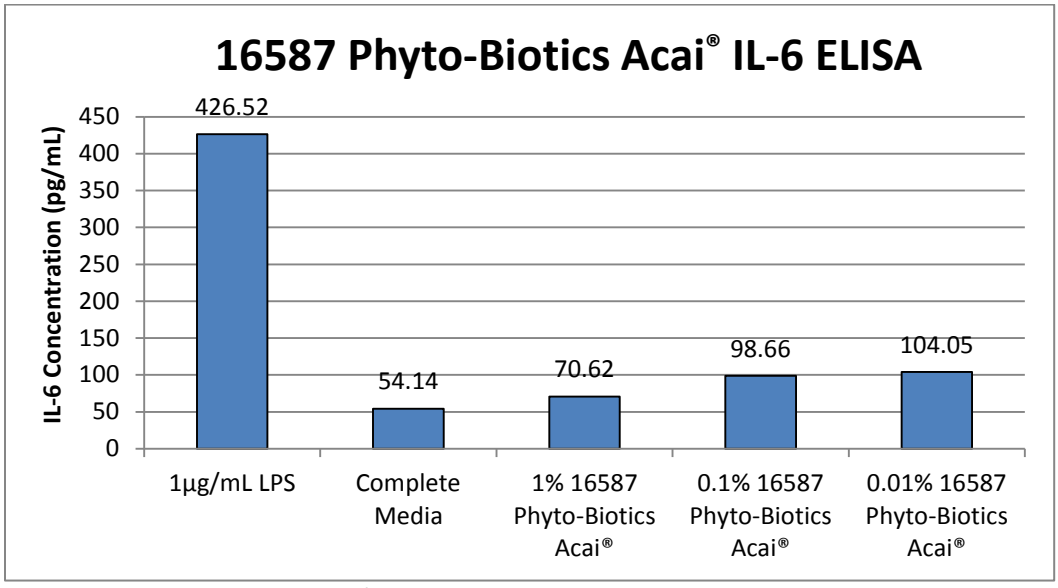

Figure 1: Phyto-Biotics Acai®-treated fibroblasts IL-6 concentrations

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

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

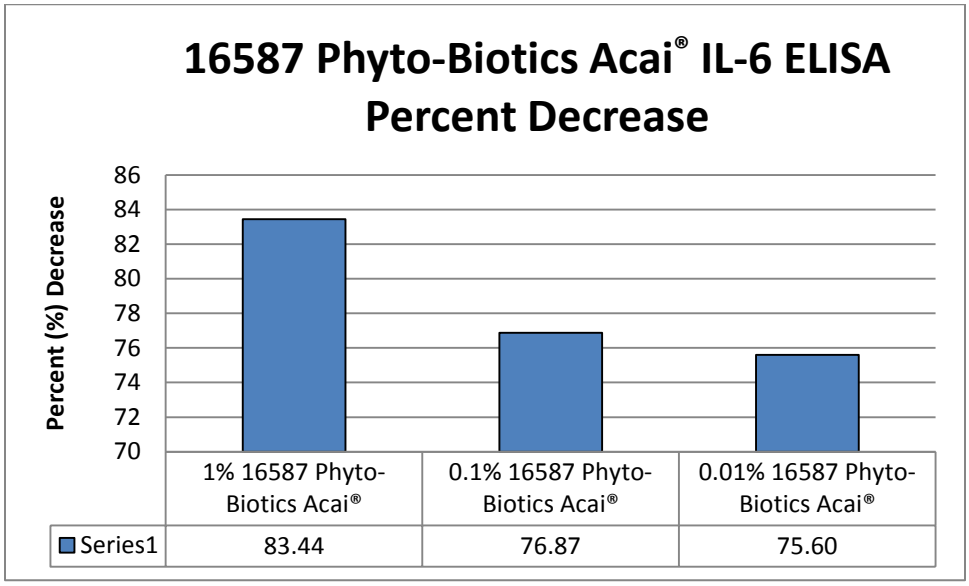

Figure 2: Percent decrease in IL-6 production compared to positive control

Discussion

As shown in figure 1, **Phyto-Biotics Acai®** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **Phyto-Biotics Acai®** 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 Acai®** is suitable for cosmetic applications designed to provide soothing and anti-aging properties.

Cellular Viability Assay Analysis

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Tradename: Phyto-Biotics Acai®

Code: 16587

CAS #: 999999-99-4

Test Request Form #: 361

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 Acai®** 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.

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Cellular Viability Assay Analysis

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 Acai**® 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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Cellular Viability Assay Analysis

Results

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

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

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

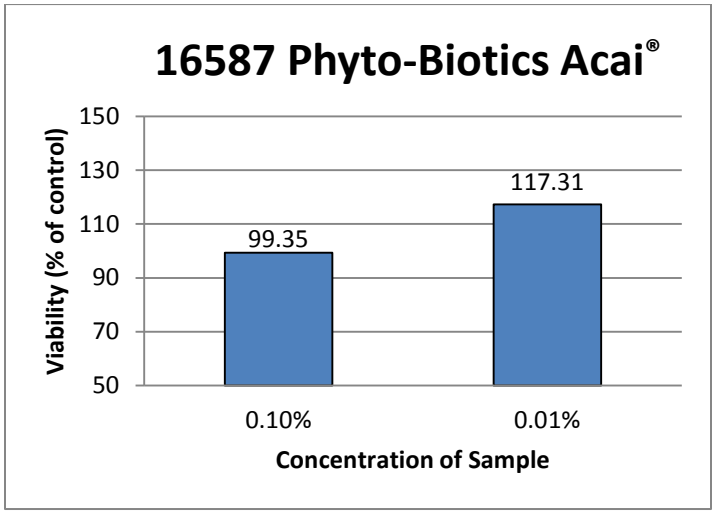

Figure 1: Cellular Metabolism of **Phyto-Biotics Acai®**-treated fibroblasts expressed in terms of percent of control.

Discussion

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

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Moisturization/Hydration Assay

Tradename: Phyto-Biotics Acai®

Code: 16587

CAS #: 999999-99-4

Test Request Form #: 494

Lot #: NC130927-A

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 **Phyto-Biotics Acai®**. 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 moisturization assay was conducted to assess the moisturizing ability of **Phyto-Biotics Acai®**.

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 4 weeks. The test material consisted of 2% **Phyto-Biotics Acai®** 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 Acai® showed very high moisturizing capabilities at a 2.0% concentration. Please note, each value is an average of three consecutive readings per test site.

Moisturization		T = 0	T= 24 Hours	T = 1 Week	T = 2 Week	T= 3 Weeks	T= 4 Weeks	T= -24 Hours	T= -1 Week
Panelist 1	Experimental	57	127	169	167	165	170	125	115
	Base Lotion	49	119	153	152	151	150	135	126
	Untreated	51	63	67	80	88	113	100	95
Panelist 2	Experimental	63	167	164	167	172	179	170	144
	Base Lotion	65	137	154	151	157	158	149	115
	Untreated	49	53	61	65	67	64	59	51
Panelist 3	Experimental	45	120	133	160	169	182	165	144
	Base Lotion	35	112	117	128	125	99	80	62
	Untreated	70	113	110	101	111	109	89	73
Panelist 4	Experimental	67	117	159	195	200	202	125	100
	Base Lotion	77	82	101	118	119	125	95	80
	Untreated	55	77	47	51	49	53	51	43
Panelist 5	Experimental	51	134	143	140	135	137	125	100
	Base Lotion	55	122	130	132	127	137	125	112
	Untreated	63	105	95	97	99	91	83	64
Panelist 6	Experimental	69	138	144	163	170	182	169	143
	Base Lotion	59	120	131	143	147	151	135	117
	Untreated	53	78	63	77	75	79	70	62
Panelist 7	Experimental	37	91	127	99	123	135	120	103
	Base Lotion	43	108	112	98	92	102	94	63
	Untreated	45	84	93	93	89	87	71	60
Panelist 8	Experimental	95	179	210	200	192	196	175	155
	Base Lotion	91	145	156	159	153	159	145	129
	Untreated	85	122	132	125	108	82	79	74
Panelist 9	Experimental	37	92	123	131	130	132	114	90
	Base Lotion	43	97	104	84	92	98	84	69
	Untreated	43	67	77	134	120	90	87	66
Panelist 10	Experimental	79	171	180	189	192	194	185	150
	Base Lotion	85	150	174	179	169	164	145	120
	Untreated	59	99	110	77	69	76	75	72
Number of Panelists		10	10	10	10	10	10	10	10

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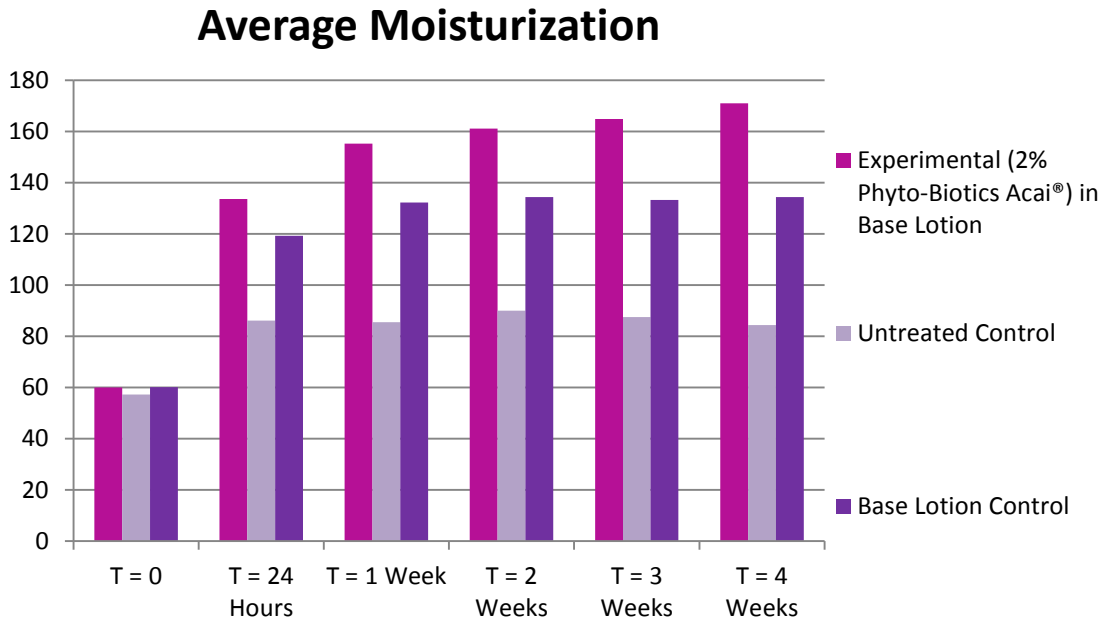
Averages	T = 0	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week
Experimental (2.0% Phyto-Biotics Acai®) in Base Lotion	60	133.6	155.2	161.1	164.8	170.9	174.3	124.4
Base Lotion Control	57.3	81.1	85.5	90	87.5	84.4	76.4	66
Untreated Control	60.2	119.2	132.2	134.4	133.2	134.3	118.7	88.

Percent (%) Change	T = 0	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 Control	5.06	38.44	55.79	49.33	52.23	59.12	55.36	50.48
Experimental vs. Untreated Control	4.71	51.17	81.52	79.00	88.34	102.48	92.80	88.48
Experimental vs. Base Lotion	0.33	12.08	16.51	19.87	23.72	27.25	24.09	25.28



Graph 1. Average moisturization levels measured at each test site.

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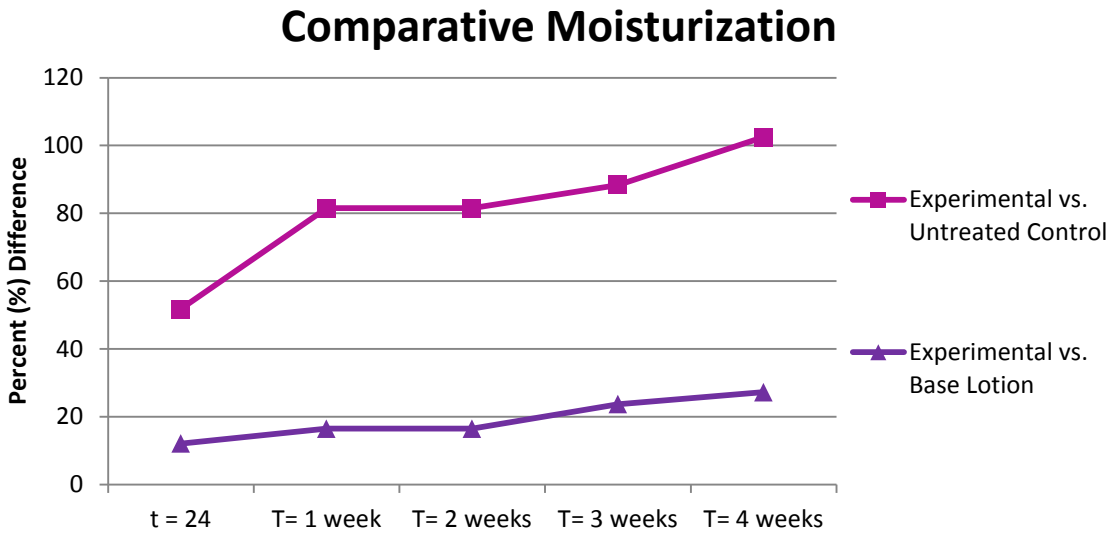
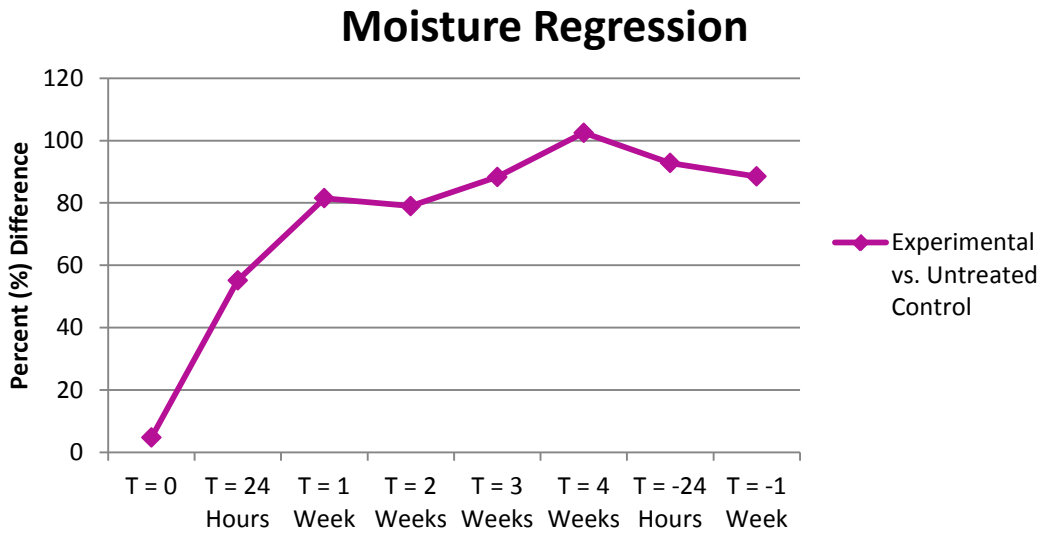


Graph 2. Comparative moisture analysis between test sites



Graph 3. Comparative analysis of moisture regression over time

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Discussion

As evidenced in a 4 week efficacy study of **Phyto-Biotics Acai®** on skin, moisture levels were improved by 51.17% after 24 hours and by 102.48% after 4 weeks when compared to the untreated control. When compared to the base cream **Phyto-Biotics Acai®** improved moisturization by 12.08% and after 4 weeks **Phyto-Biotics Acai®** improved moisturization by 27.25%. Results indicate that **Phyto-Biotics Acai®** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

The present study confirms that **Phyto-Biotics Acai®** is not only capable of providing functional benefits but it is also capable of providing moisturizing and skin hydrating benefits when added to cosmetic applications.