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Tomorrow's Vision... *Today!®*

Phytofuse Rejuvenate® Efficacy Data

Code: 16882
INCI Name: Salvia Hispanica Seed Extract
CAS #: 93384-40-8
EINECS #: 297-250-8

Type of Study	Results
Cellular Viability Assay	At concentrations of 1%, 0.1%, and 0.01% **Phytofuse Rejuvenate®**, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **Phytofuse Rejuvenate®** is not cytotoxic.
IL-6 ELISA Analysis	**Phytofuse Rejuvenate®** exhibited anti-inflammatory effects on LPS-treated fibroblasts. 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. It can therefore be concluded that at normal use concentrations **Phytofuse Rejuvenate®** enhances soothing and anti-aging properties.
High Resolution Ultrasound Skin Imaging Assay	As evidenced in a 4 week efficacy study of **Phytofuse Rejuvenate®** on skin, skin density was improved by 5.44% after 24 hours and by 28.03% after 4 weeks when compared to the untreated control. When compared to the base cream **Phytofuse Rejuvenate®** improved skin density by 6.29% after 24 hours and after 4 weeks **Phytofuse Rejuvenate®** improved density by 23.93%. Results indicate that **Phytofuse Rejuvenate®** is capable of improving skin density when compared to both the untreated control as well as the base lotion. **Phytofuse Rejuvenate®** has a positive effect on skin's density when used at recommended use levels.
Moisturization Assay	As evidenced in a 4 week efficacy study of **Phytofuse Rejuvenate®** on skin, moisture levels were improved by 36.96% after 24 hours and by 120.89% after 4 weeks when compared to the untreated control. Comparisons of the base lotion to the Experimental Lotion containing 2.0% **Phytofuse Rejuvenate®** demonstrate the experimental material moisturized the skin 9.32% better after 24 hours. After four weeks the base lotion containing 2.0% **Phytofuse Rejuvenate®** moisturized skin 35.98% better than the base lotion alone. Results indicate that **Phytofuse Rejuvenate®** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

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ORAC Assay	**Phytofuse Rejuvenate®** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **Phytofuse Rejuvenate®** increased as the concentration increased. As a result we can assure that its ability to minimize oxidative stress is dose dependent. It can therefore be concluded that **Phytofuse Rejuvenate®** is capable of providing antioxidant properties.
Scratch Assay	**Phytofuse Rejuvenate®** was able to increase cell migration and close the scratch at a rate comparable to the positive control. The mechanisms of the cells in the in vitro scratch assay mimic the mechanisms seen in *in vivo* wound healing therefore we can be assured that our results are translatable outside the laboratory. With the present study we can be confident that this product has healing abilities and cell proliferation properties.
Transepidermal Water Loss Study	After one week, the solution containing 2.0% **Phytofuse Rejuvenate®** decreased TEWL 9.23% more effectively than the base lotion alone. After three weeks, the solution containing 2.0% **Phytofuse Rejuvenate®** demonstrated even more effective barrier protection, decreasing TEWL 27.14% better than the base lotion alone. Results indicate that **Phytofuse Rejuvenate®** is capable of reducing TEWL, which allows for moisture retention.
Anti-Pollution Assay Analysis	**Phytofuse Rejuvenate®** was able to provide pollution protection as specified by micronized carbon residue. The small amount of carbon that remains compared to the untreated control indicates the ability of **Phytofuse Rejuvenate®** to provide barrier protection against everyday air pollution and slow the extrinsic aging process. It can therefore be concluded that at normal use concentrations **Phytofuse Rejuvenate®** can be used as a skin pollution protection active ingredient.

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Cellular Viability Assay Analysis

Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 897

Lot #: NC140813-H

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 **Phytofuse Rejuvenate®** 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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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 **Phytofuse Rejuvenate**® 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 and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

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Results

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

Phytofuse Rejuvenate® did not have negative effects on cell metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$Percent\ (\%)Change = \frac{MFU_{Control} - MFU_{Sample}}{MFU_{Control}} \times 100$$

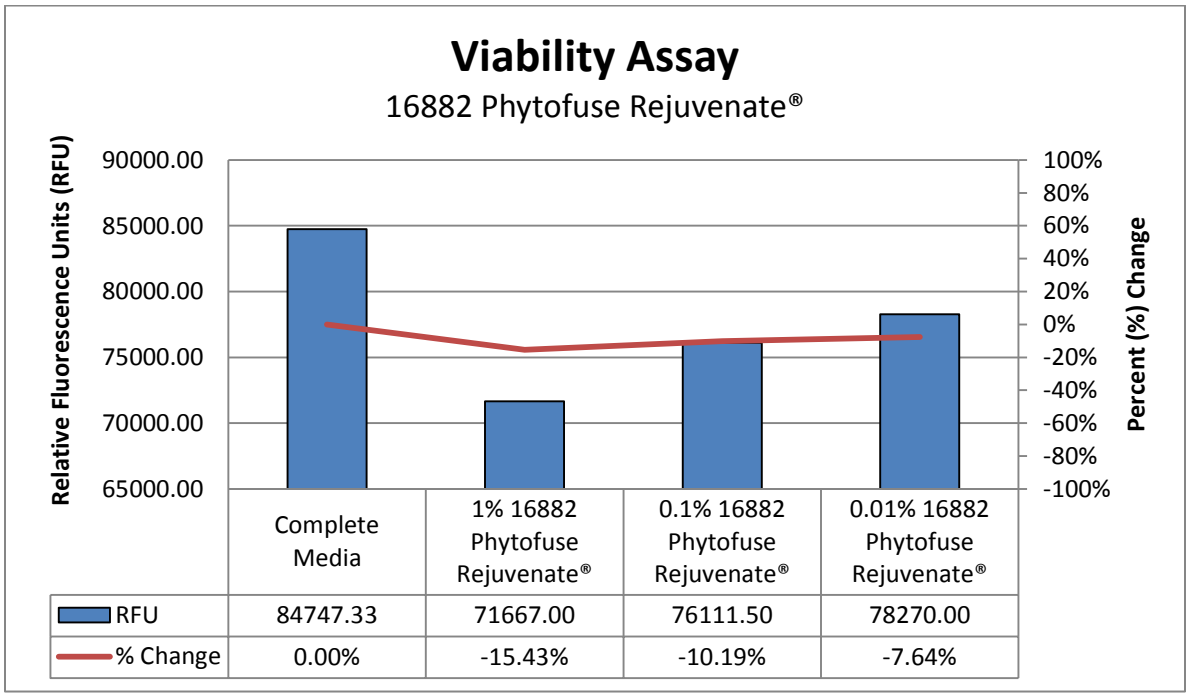

Figure 1: Cellular Metabolism of **Phytofuse Rejuvenate**®-treated fibroblasts expressed in terms of percent of control.

Discussion

In this study, **Phytofuse Rejuvenate**® (code 16882) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of 1%, 0.1%, and 0.01% **Phytofuse Rejuvenate**®, nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **Phytofuse Rejuvenate**® is not cytotoxic.

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IL-6 ELISA Analysis

Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 899

Lot #: NC140813-H

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 **Phytofuse Rejuvenate®**-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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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); Dexamethasone (10µM)
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 **Phytofuse Rejuvenate**® were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 72 hours. Complete media containing 1µg/mL LPS was used to create an inflammatory environment and dexamethasone (DEX) in the presence of LPS was used as a positive control to quell inflammation.

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 **Phytofuse Rejuvenate**® 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.

Phytofuse Rejuvenate® at a concentration of 1% was able to decrease IL-6 production.

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

$$Percent\ (\%)\ Change = \frac{IL\ 6\ Concentration_{Sample} - IL\ 6\ Concentration_{1\mu M/mL\ LPS}}{IL\ 6\ Concentration_{1\mu M/mL\ LPS}} \times 100$$

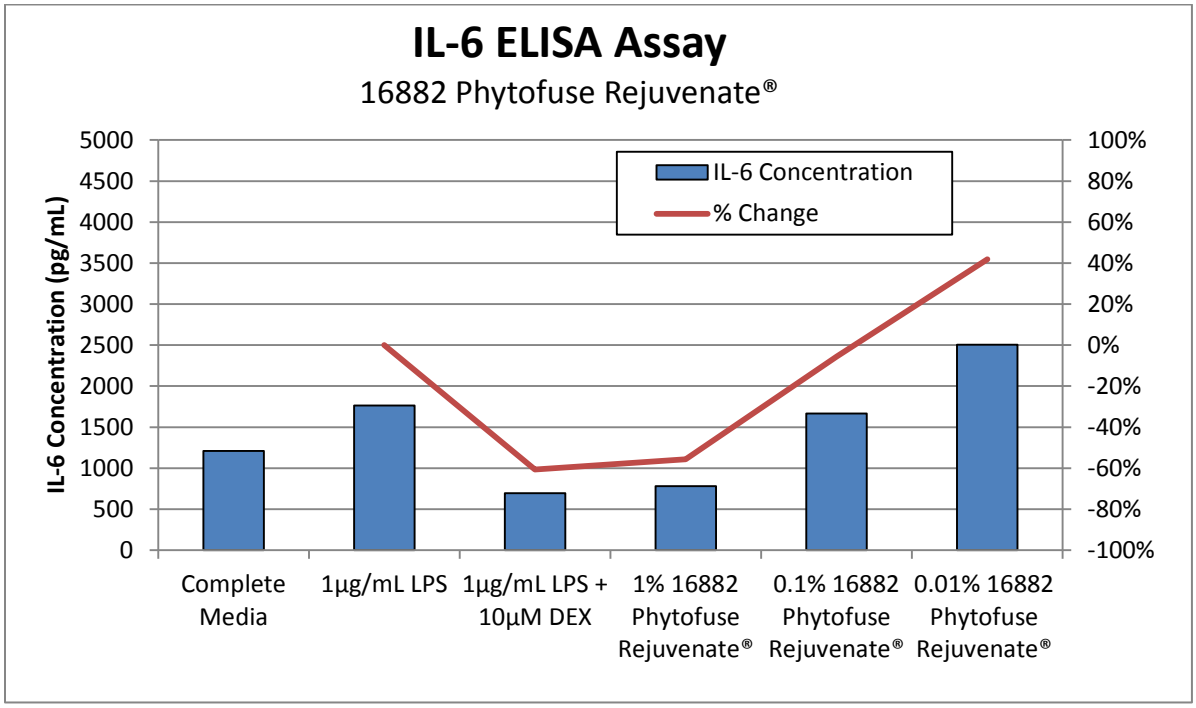

Figure 1: Phytofuse Rejuvenate®-treated fibroblasts IL-6 concentrations and percent change

Discussion

As shown in figure 1, **Phytofuse Rejuvenate**® (code 16882) exhibited anti-inflammatory effects on LPS-treated fibroblasts. 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. It can therefore be concluded that at normal use concentrations **Phytofuse Rejuvenate**® enhances soothing and anti-aging properties.

High Resolution Ultrasound Skin Imaging Assay

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Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 995

Lot #: NC140105-A

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

Test Performed:
High Resolution Ultrasound Skin-Imaging Assay

Introduction

An *in-vivo* study was conducted over a period of four weeks to evaluate the effect on skin density of **Phytofuse Rejuvenate®.** Ten M/F subjects between the ages of 23-45 participated in the study. Data gathered from the high resolution ultrasound imaging yielded results that indicate that this material is capable of significantly improving skin density compared to the control.

Materials

A. Equipment: DermaLab Skin Combo (Ultrasound Probe)

Methods

High Resolution Ultrasound Skin imaging is based on measuring the acoustic response after an acoustic pulse is sent into the skin. The energy of the acoustic pulse is low and will not affect the skin in any way. When the acoustic pulse is emitted and hits different areas of the skin, part of the pulse will be reflected and part will be transmitted further into the skin. The reflected signal travels back and is picked up by the ultrasound transducer. After processing the signal, a cross-sectional image appears on the screen. This image represents an intensity, or amplitude, analysis of the signals.

The intensity of the signals that are received refer to a color scale. Dark colors represent areas of the skin with low reflection. This means that there are no changes or very small changes in density between the structures in the skin. Bright colors represent areas with strong reflections, signifying substantial changes in density between structures.

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Ten 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. The DermaLab ultrasound probe was used to determine the skin density of the subject's volar forearms.

Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken 24 hours after application of test materials and then weekly for 4 weeks. The test material consisted of 2.0% **Phytofuse Rejuvenate®** 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

Phytofuse Rejuvenate® showed improvements in skin density at a 2.0% concentration. Please note, each value is an average of three consecutive readings per test site.

Ultrasound		T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks
Panelist 1	Experimental	45	53	53	60	68
	Base Lotion	60	61	55	59	60
	Untreated	74	71	68	70	72
Panelist 2	Experimental	75	78	75	78	82
	Base Lotion	76	80	78	77	75
	Untreated	60	60	53	55	61
Panelist 3	Experimental	63	58	56	65	70
	Base Lotion	60	68	70	73	77
	Untreated	65	63	57	60	62
Panelist 4	Experimental	73	97	100	112	130
	Base Lotion	64	66	64	68	70
	Untreated	50	66	65	59	60
Panelist 5	Experimental	60	70	71	75	76
	Base Lotion	61	62	63	65	67
	Untreated	71	72	75	76	79
Panelist 6	Experimental	72	62	62	72	75
	Base Lotion	50	58	57	59	65
	Untreated	27	28	36	35	34
Panelist 7	Experimental	81	84	92	100	100
	Base Lotion	45	50	53	51	53
	Untreated	88	90	85	90	83
Panelist 8	Experimental	52	52	54	55	56
	Base Lotion	79	77	74	77	73
	Untreated	58	55	53	46	56
Panelist 9	Experimental	81	84	87	89	90
	Base Lotion	67	64	66	68	65
	Untreated	75	74	75	73	82
Panelist 10	Experimental	57	59	62	63	66
	Base Lotion	58	52	45	45	51
	Untreated	57	55	41	43	46
Number of Panelists		10	10	10	10	10

Figure 1: Individual Raw Data

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	T = 24	1 week	2 week	3 week	4 week
Experimental (2.0% Phytofuse Rejuvenate® in Base Lotion)	65.9	69.7	71.2	76.9	81.3
Untreated Control	62.5	63.4	60.8	60.7	63.5
Base Lotion Control	62.0	63.8	62.5	64.2	65.6

Figure 2: Average values

	T = 24	1 week	2 week	3 week	4 week
Base Lotion vs. Untreated	-0.80%	0.63%	2.80%	5.77%	3.31%
Experimental vs. Untreated	5.44%	9.94%	17.11%	26.69%	28.03%
Experimental vs. Base Lotion	6.29%	9.25%	13.92%	19.78%	23.93%

Figure 3: Percent change

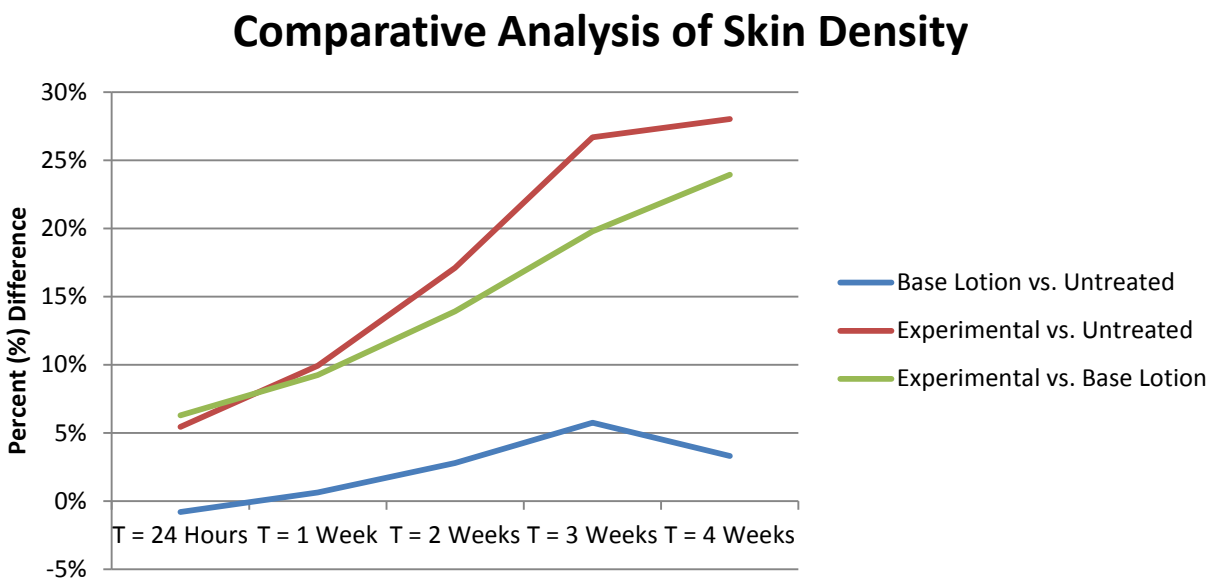

Figure 4: Percent difference in skin density recordings between test materials

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Discussion

As evidenced in a 4 week efficacy study of **Phytofuse Rejuvenate®** on skin, skin density was improved by 5.44% after 24 hours and by 28.03% after 4 weeks when compared to the untreated control. When compared to the base cream **Phytofuse Rejuvenate®** improved skin density by 6.29% after 24 hours and after 4 weeks **Phytofuse Rejuvenate®** improved density by 23.93%. Results indicate that **Phytofuse Rejuvenate®** is capable of improving skin density when compared to both the untreated control as well as the base lotion.

Phytofuse Rejuvenate® has a positive effect on skin's density when used at recommended use levels.

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Moisturization/Hydration Assay

Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 995

Lot #: NC140105-A

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

Test Performed:
Moisturization/ Hydration Assay

Introduction

An *in-vivo* study was conducted over a period of four weeks to evaluate the moisturization benefits of **Phytofuse Rejuvenate**®. 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 **Phytofuse Rejuvenate**®.

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.

Ten 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 24 hours after application of test materials and then weekly for 4 weeks. The test material consisted of 2.0% **Phytofuse Rejuvenate**® 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

Phytofuse Rejuvenate® 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 = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week	T = -2 Weeks
Panelist 1	Experimental	118	150	211	229	235	200	116	100
	Base Lotion	109	115	192	184	191	115	77	75
	Untreated	91	99	97	101	100	97	98	80
Panelist 2	Experimental	153	200	261	280	350	300	140	100
	Base Lotion	185	200	250	250	300	175	160	110
	Untreated	110	106	92	100	105	110	109	95
Panelist 3	Experimental	150	259	300	310	340	270	200	180
	Base Lotion	105	200	216	210	225	200	150	140
	Untreated	120	209	215	200	230	170	160	150
Panelist 4	Experimental	118	272	280	290	300	210	100	75
	Base Lotion	160	193	190	179	180	150	77	50
	Untreated	72	117	110	73	90	85	70	60
Panelist 5	Experimental	125	190	198	207	219	140	77	60
	Base Lotion	100	173	168	165	150	110	60	55
	Untreated	120	118	164	170	122	115	109	100
Panelist 6	Experimental	96	150	184	210	280	200	75	45
	Base Lotion	80	173	168	160	165	100	86	70
	Untreated	67	129	150	155	175	95	80	75
Panelist 7	Experimental	187	201	220	243	269	200	116	84
	Base Lotion	99	124	155	184	191	115	83	79
	Untreated	110	116	124	139	130	117	95	90
Panelist 8	Experimental	171	194	199	212	232	165	140	130
	Base Lotion	185	200	250	250	300	175	160	154
	Untreated	112	108	106	100	105	108	109	102
Panelist 9	Experimental	172	194	254	310	340	250	200	175
	Base Lotion	105	200	216	210	225	200	150	140
	Untreated	108	100	95	106	108	95	87	85
Panelist 10	Experimental	118	272	280	290	300	210	100	75
	Base Lotion	160	193	190	179	180	150	77	80
	Untreated	118	117	120	125	132	128	119	111
Number of Panelists		10	10	10	10	10	10	10	10

Chart 1. Panelist Moisturization Measurements

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Averages	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week
2.0% Phytofuse Rejuvenate® in Base Lotion	140.8	208.2	238.7	258.1	286.5	214.5	126.4
Base Lotion	128.8	177.1	199.5	197.1	210.7	149.0	108.0
Untreated	102.8	121.9	127.3	126.9	129.7	112.0	103.6

Chart 2. 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	25.29	45.28	56.72	55.32	62.45	33.04	4.25
2.0% Phytofuse Rejuvenate® + Base Lotion vs. Untreated	36.96	70.79	87.51	103.34	120.89	91.52	22.01
2.0% Phytofuse Rejuvenate® in Base Lotion vs. Base Lotion	9.32	17.56	19.65	30.95	35.98	43.96	17.04

Chart 3. Comparative Moisture Increase and Regression Scores Between Individual Test Sites

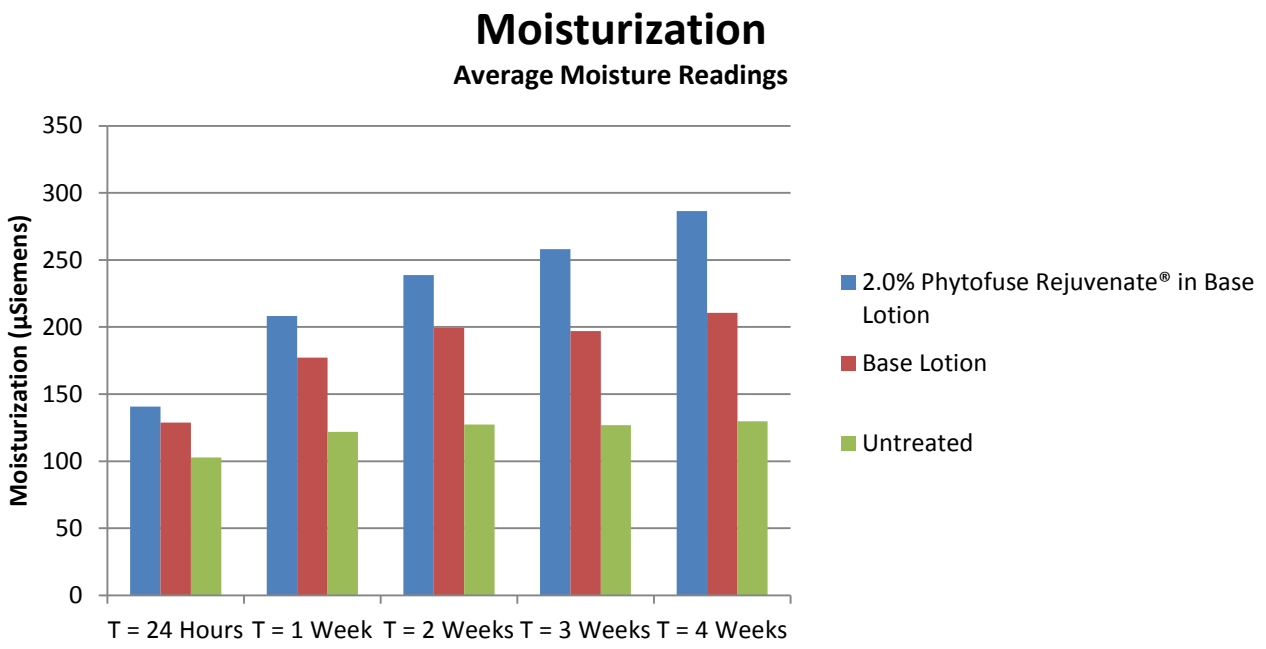

Figure 1. Average increase in moisturization per test site

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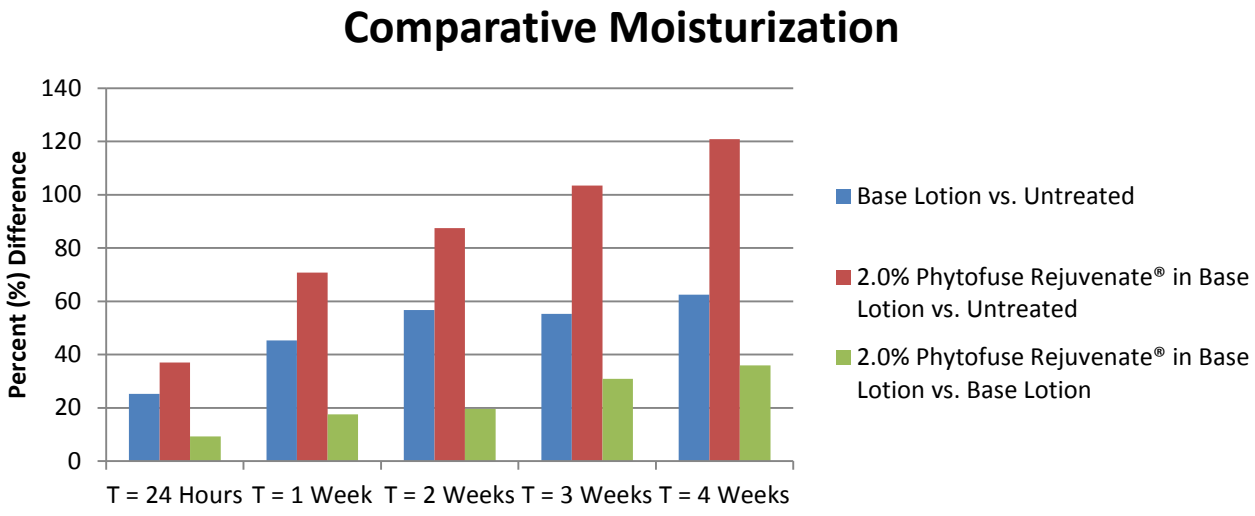

Figure 2. Percent difference in moisturization between two test sites over four weeks

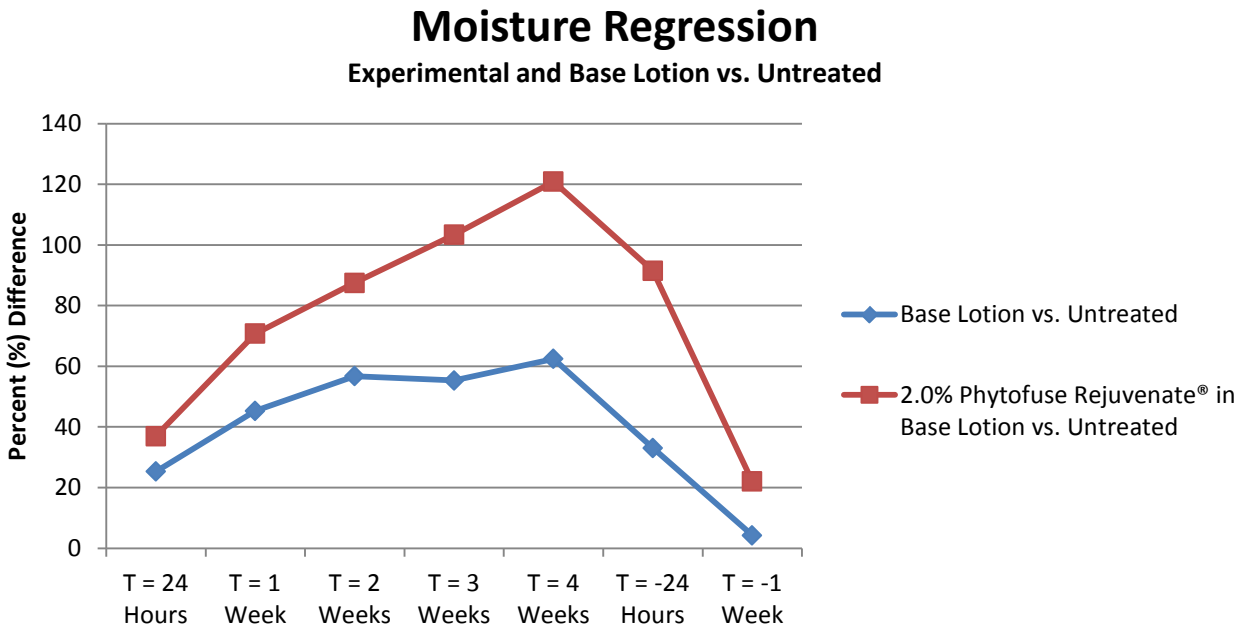

Figure 3. Regression in skin moisturization after application of experimental and base lotion material ceased

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Discussion

As evidenced in a 4 week efficacy study of **Phytofuse Rejuvenate®** on skin, moisture levels were improved by 36.96% after 24 hours and by 120.89% after 4 weeks when compared to the untreated control. Comparisons of the base lotion to the Experimental Lotion containing 2.0% **Phytofuse Rejuvenate®** demonstrate the experimental material moisturized the skin 9.32% better after 24 hours. After four weeks the base lotion containing 2.0% **Phytofuse Rejuvenate®** moisturized skin 35.98% better than the base lotion alone. Results indicate that **Phytofuse Rejuvenate®** 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 **Phytofuse Rejuvenate®** 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% **Phytofuse Rejuvenate® + Base Lotion** was approximately 91.52% more moisturized than the site which did not receive treatment. After one week, the experimental test site was still yielding moisturization results that were 22.01% higher than the untreated site. Additionally, in comparison to the site tested with the base lotion alone, the site treated with 2.0% **Phytofuse Rejuvenate® + Base Lotion** moisturized the skin 43.96% better after 24 hours after and was still 17.04% more effective in moisturizing the skin when reading were taken one week after the applications of both test materials ceased.

Phytofuse Rejuvenate® was designed to provide moisturization benefits, however with the present study we can confirm that this ingredient is not only capable of providing protective benefits but also ideal for moisturizing and skin hydrating personal care applications.

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 896

Lot #: NC150119-B

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 **Phytofuse Rejuvenate®**.

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 **Phytofuse Rejuvenate**® 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.

ORAC values are also calculated in Units/milliliter (U/mL). The equation used for the calculation is shown below:

$$ORAC\ (U/mL) = (50 \times Dilution\ Factor) \times \left(\frac{AUC_{Sample} - AUC_{Blank}}{AUC_{Trolox} - AUC_{Blank}}\right)$$

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Results

Phytofuse Rejuvenate® began exhibiting antioxidant activity at a 1.25% concentration.

The ORAC value expressed in U/mL for 1.25% **Phytofuse Rejuvenate®** is 3256.8.

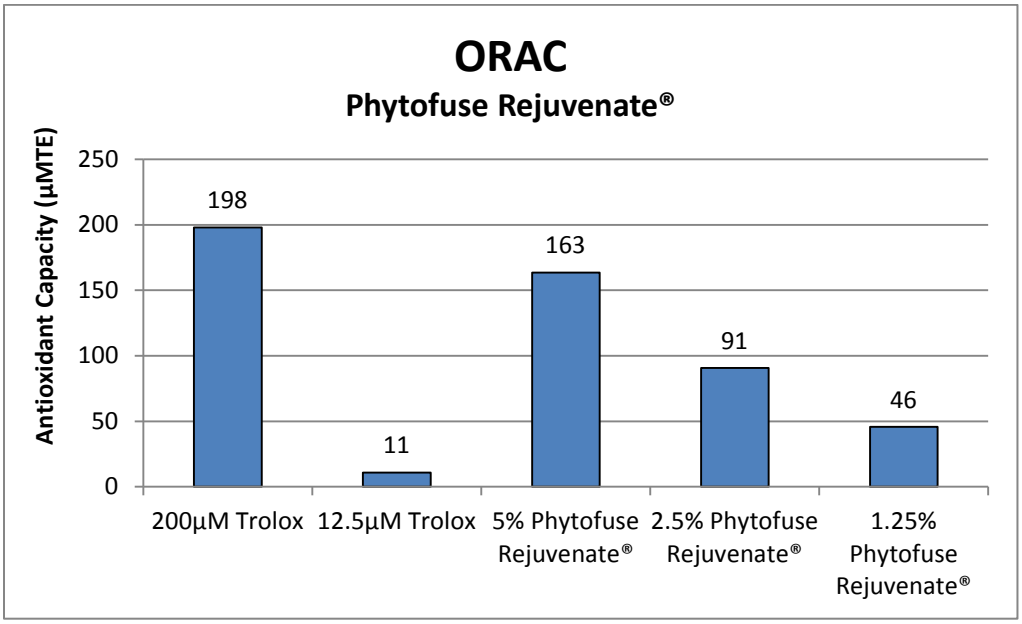

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **Phytofuse Rejuvenate®** (code 16882) exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **Phytofuse Rejuvenate®** increased as the concentration increased. As a result we can assure that its ability to minimize oxidative stress is dose dependent. It can therefore be concluded that **Phytofuse Rejuvenate®** is capable of providing antioxidant properties.

Active Concepts

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Scratch Assay Analysis

Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 900

Lot #: NC150119-B

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

Test Performed:
Scratch Assay

Introduction

Wounded tissue begins a complex and structured series of events in order to repair the damaged region. Some of these events include upregulation of angiogenic factors causing increased vascularization, increased deposition of extracellular matrix, and increased cell proliferation. The wound healing process begins as cells polarize toward the wound, initiate protrusion, migrate, and close the wound area. These processes reflect the behavior of individual cells as well as the entire tissue complex.

The scratch assay was conducted to assess the wound healing properties of **Phytofuse Rejuvenate®-**treated *in vitro* cultured human dermal fibroblasts.

Assay Principle

The *in vitro* scratch assay is a well-known and widely used method to study cell migration and proliferation. This assay is based on the observation that when an artificial gap or scratch is made on a confluent cell monolayer, the cells will migrate towards the opening and close the scratch. The basic steps involve creating a scratch in a cell monolayer and capturing images throughout the healing or cell migration process. Through these images we can quantify the rate of cell migration.

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Materials

A. Incubation Conditions:	37°C at 5% CO_2 and 95% Relative Humidity (RH)
B. Equipment:	Forma Humidified Incubator, ESCO Biosafety Laminar Flow Hood, Inverted Microscope; Camera; Pipettes
C. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
D. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Fetal Bovine Serum (FBS); Penicillin-Streptomycin (50U-50mg/mL); Phosphate Buffered Saline (PBS)
E. Reagents:	Epidermal Growth Factor-1 (100ng/mL); Paraformaldehyde (3.7%); Crystal Violet Stain
F. Culture Plate:	Falcon Flat Bottom 6-Well Tissue Culture Treated Plates
G. Other:	Sterile Disposable Pipette Tips; Wash Bottles; 15mL Conical Tubes

Methods

Human dermal fibroblasts were seeded into 6-well tissue culture plates and allowed to grow to confluency in complete DMEM. 0.1% and 0.2% concentrations of **Phytofuse Rejuvenate®** was added to the culture media and incubated with fibroblasts for the extent of the experiment. Epidermal Growth Factor-1 was utilized as the positive control and serum-free media (SFM) was used a negative control. Complete media contains 10% FBS.

When cell growth reached confluency scratches were made across the well in a cross or 'X' pattern. The wells were washed with sterile PBS and fresh media containing **Phytofuse Rejuvenate®** and the controls were added. Initial images were captured immediately after the scratch took place and every 24-hours afterwards, up to 72-hours. Cells were fixed with 3.7% paraformaldehyde and stained with crystal violet for enhanced microscopy.

ImageJ software was used to analyze the images and calculate the area of the scratch and the closure rate.

Results

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

Phytofuse Rejuvenate® at concentrations of 0.1% and 0.2% were able to increase cell migration and wound healing compared to our negative control.

Percent scratch closure and migration rate are expressed by the following formula:

$$\frac{Scratch\ Area_{\ t=x} - Scratch\ Area_{\ t=0}}{Scratch\ Area_{\ t=0}} \times 100 = \%\ Scratch\ Closure$$

$$\frac{Change\ in\ Area\ of\ Scratch\ (nm^2)}{Migration\ Time_{\ t=x}} = Migration\ Rate$$

$$Where\ x = time\ (hours)\ post\ scratch$$

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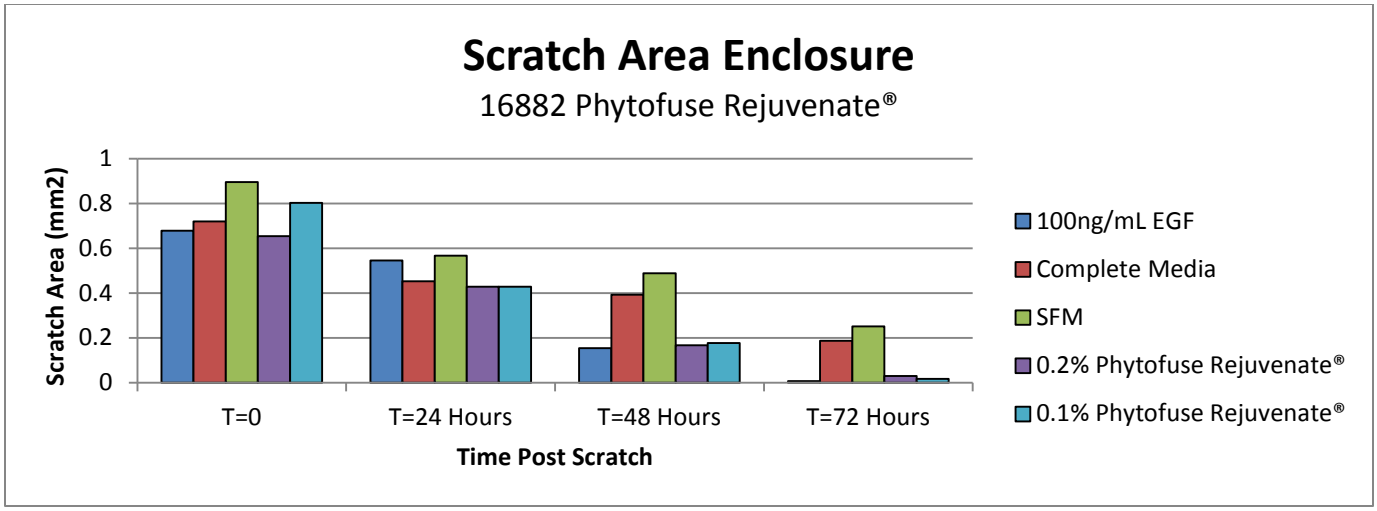

Figure 1: Area of scratch

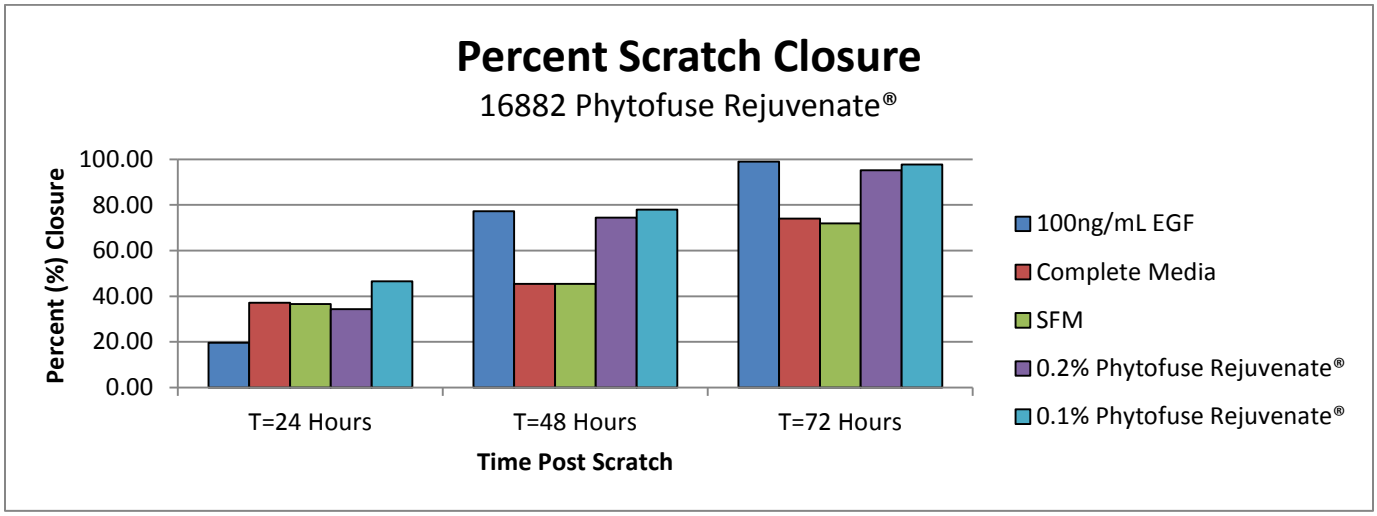

Figure 2: Percent scratch closure

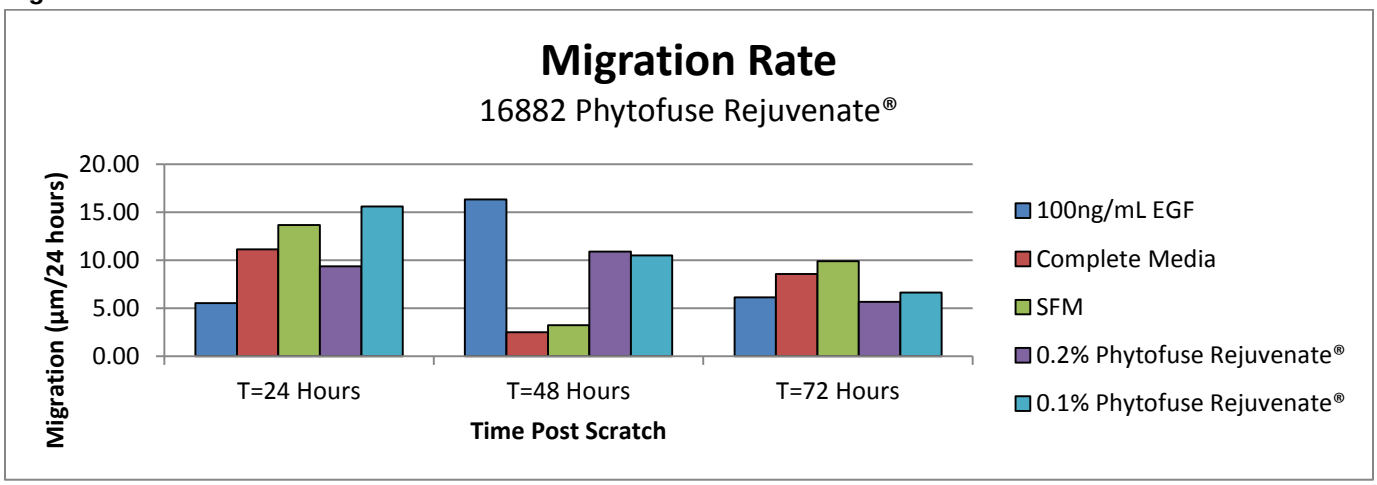

Figure 3: Cell migration rate

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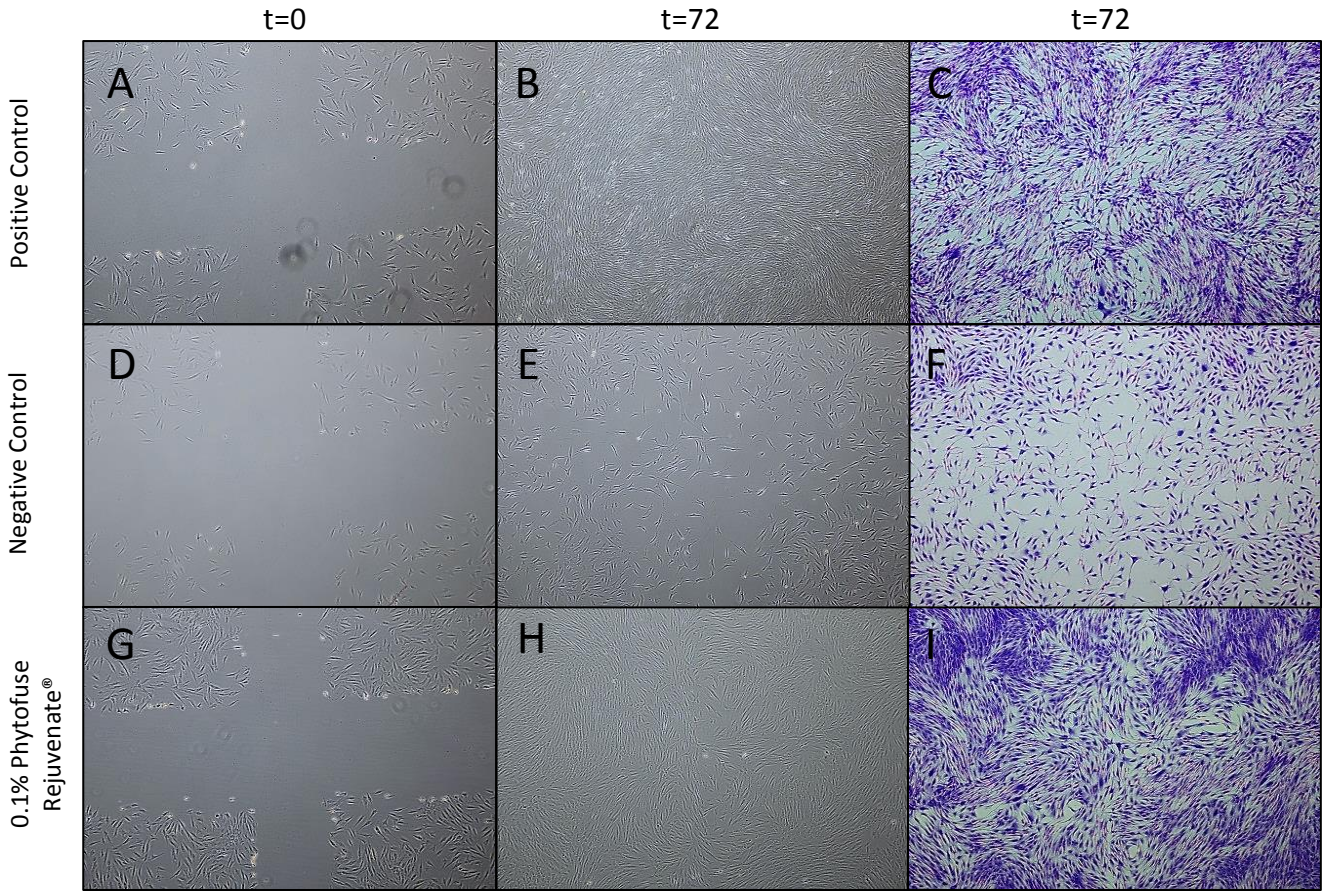

Figure 4: Images at t=0 hours (A, D, G) and t=72 hours (B, E, H) for **Phytofuse Rejuvenate®**, positive control (EGF-1), and negative control (SFM). At experiment completion (t=72 hours), cells were fixed in paraformaldehyde and stained with crystal violet (C, F, I).

Discussion

Phytofuse Rejuvenate® (code 16882) was able to increase cell migration and close the scratch at a rate comparable to the positive control. The mechanisms of the cells in the *in vitro* scratch assay mimic the mechanisms seen in *in vivo* wound healing therefore we can be assured that our results are translatable outside the laboratory. With the present study we can be confident that this product has healing abilities and cell proliferation properties.

Transepidermal Water Loss Study

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Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 995

Lot #: NC140105-A

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

Test Performed:
Transepidermal Water Loss Study

Introduction

An *in-vivo* study was conducted over a period of three weeks to evaluate the ability of **Phytofuse Rejuvenate®** to enhance barrier function through reduction in Transepidermal Water Loss (TEWL). Results indicate that this material is capable of efficiently reducing TEWL which allows moisture retention.

Materials

A. Equipment: DermaLab Skin Combo

Methods

Ten 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 Combo was used to measure TEWL on the subject's volar forearms. The instrument consists of a probe that is based upon the vapor gradient with an open chamber. This open chamber design maintains the free natural evaporation from the skin without interfering with the environment over the measurement area. This ensures unbiased and accurate readings. Operation of the water loss module is fully menu drive, allowing for pre-setting and standard deviation or measurement time. Baseline TEWL readings were taken on day one of the study.

Following initial measurements, all subjects were asked to apply 5 milligrams of each test material on their volar forearms. Measurements were taken 24 hours after application of the test materials and then weekly for four weeks. The test material consisted of 2.0% **Phytofuse Rejuvenate®** 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.

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Results

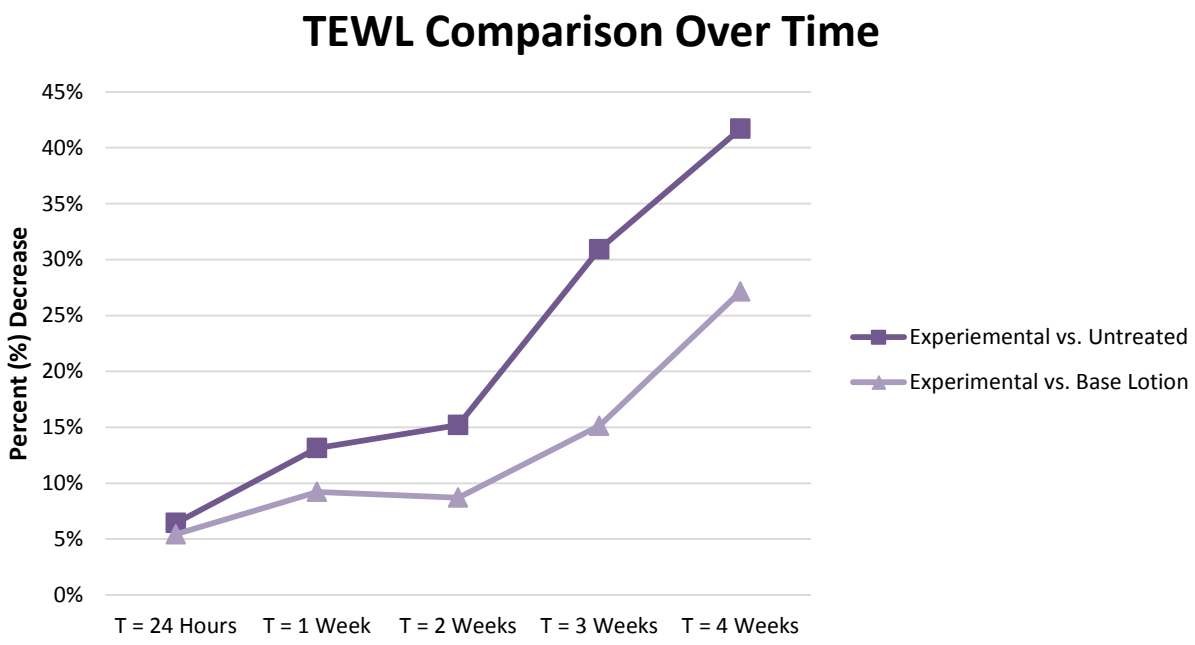

Figure 1: Improvements in barrier function following application of the test materials after a period of 4 weeks.

Discussion

As shown in Figure 1, results indicate continuous improvements in the barrier of the skin throughout the 4 week test period. After one week, the solution containing 2.0% **Phytofuse Rejuvenate®** decreased TEWL 9.23% more effectively than the base lotion alone. After three weeks, the solution containing 2.0% **Phytofuse Rejuvenate®** demonstrated even more effective barrier protection, decreasing TEWL 27.14% better than the base lotion alone.

Anti-Pollution Assay Analysis

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Tradename: Phytofuse Rejuvenate®

Code: 16882

CAS #: 93384-40-8

Test Request Form #: 1924

Lot #: NC160330-C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Danaher*
Principle Investigator: *Jennifer Goodman*

Test Performed:
Pollution Protection Assay

Introduction

The role of pollution in the appearance of the premature wrinkles and age spots has because a new frontier in antiaging active ingredients. While we have known about the harmful effects of pollution on our health for years, new research indicates air pollution plays a detrimental role in extrinsic aging. Carbon and metal micro particles found in polluted air embed in the dermis causing oxidative stress, initiating the inflammatory cascade leading to the breakdown on the collagen, elastin, and other structural components in the skin. Additionally, polyaromatic hydrocarbons overstimulate the aryl hydrocarbon receptors on keratinocytes and melanocytes resulting in the hyperpigmentation and the appearance of age spots. Providing a physical barrier will prevent embedment of carbon particles, thus reducing the signs extrinsic aging.

Our pollution protection assay was conducted to assess the ability of **Phytofuse Rejuvenate®** to provide immediate protection from carbon air pollution.

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Materials

A. Equipment:	Dissecting microscope; Digital camera; Pipettes
B. Reagents:	Micronized activated charcoal; Cetaphil Moisturizing for All Skin Types
C. Other:	Disposable pipette tips; wash bottles

Methods

Volunteers, male and female, between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. All subjects were asked to apply 2 mg of each test material, experimental, control, and untreated on their volar forearms. Lotions were allowed to dry completely before the addition of 5 mg of micronized charcoal. The micronized charcoal used has a particle size of 2.5 microns (PM 2.5) or less that mimics the small particulates found in polluted air. Each treatment area was washed five times using deionized water. Images were taken pre- and post-wash using a dissecting microscope.

The test material consisted of 2% **Phytofuse Rejuvenate®** in a Cetaphil Moisturizing for All Skin Types. For added perspective, images of an untreated test site and a site treated with Cetaphil Moisturizing for All Skin Types were recorded.

Color analysis was conducted on the images and results depicted in optical density values and pigmentation histograms. Images were inverted and standard coloration values recorded and assigned absorbance units. The lower the mean optical density value the better protection against carbon particle embedment or PM 2.5 inhibition.

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Results

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

Phytofuse Rejuvenate® at a concentration of 2% was able to provide protection from carbon pollution.

Figure 1: Phytofuse Rejuvenate® Histogram Images - Inhibition on PM 2.5

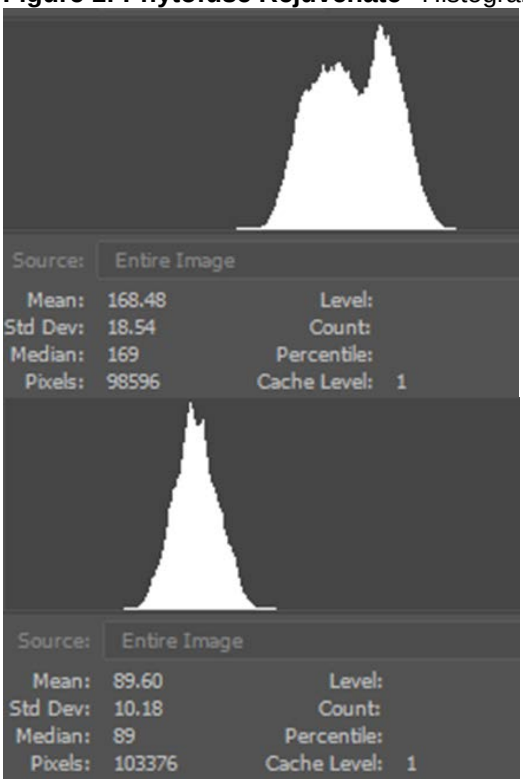

Figure 2: **Phytofuse Rejuvenate®** Images

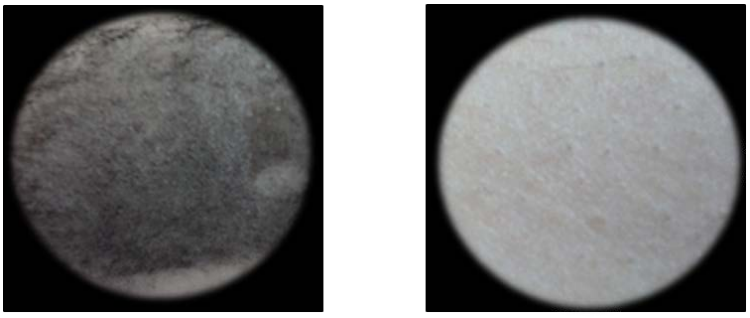

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Figure 3: Untreated Histogram Images - Inhibition on PM 2.5

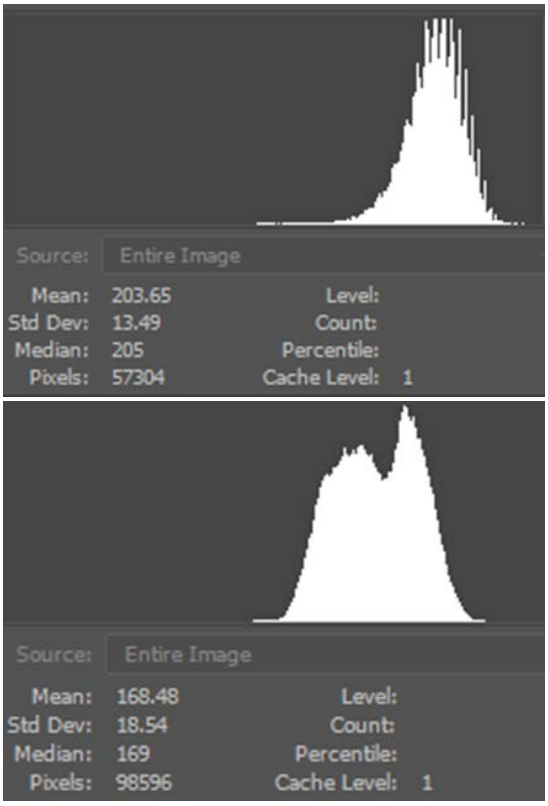
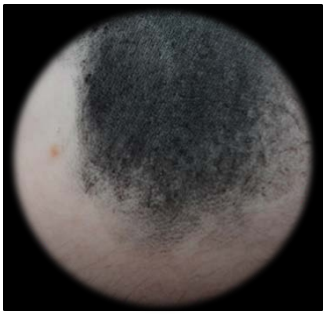
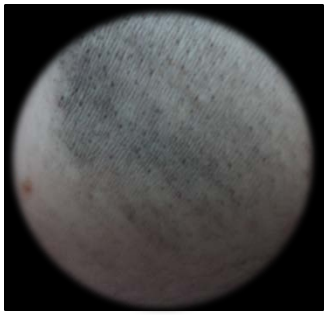


Figure 4: Untreated Images





Discussion

As shown in figure 1, **Phytofuse Rejuvenate® (code 16882)** was able to provide pollution protection as specified by micronized carbon residue. The small amount of carbon that remains compared to the untreated control indicates the ability of **Phytofuse Rejuvenate®** to provide barrier protection against everyday air pollution and slow the extrinsic aging process. It can therefore be concluded that at normal use concentrations **Phytofuse Rejuvenate®** can be used as a skin pollution protection active ingredient.