

Phyto-Biotics Acai®



stem cell technology
leuconostoc sp.
Brand Differentiation
meristemetic specific activity
anti-aging, sustainable
hair + skin care, biotic stress

BACKGROUND

The stem cell trend is here to stay. Research shows that plant stem cells can slow down aging of the skin by defending against extrinsic stress. **Phyto-Biotics Acai®** capitalizes on specific activity of phenolic metabolites produced by the Acai palms to provide a plant-derived stem cell ingredient that imparts antioxidant, protectant and powerful anti-aging benefits for an everlasting appearance. Active Concepts has chosen to use a novel approach, which focuses on biotic stress, embodied by the sustainable practice of co-culturing of plant stem cells to produce secondary metabolites. This technology also induces cellular differentiation and provides potent cosmetic benefits to any formulation.

SCIENCE

Interest in stem cell technology was once limited to medical research where scientists investigated cures for diabetes, Parkinson's disease and AIDS. Intense media coverage of the controversy surrounding stem cell research pushed the technology into mainstream focus and resulted in a growing consumer-base searching for cosmetics that made stem cell technology claims.

The "new age of anti-aging" is how Cosmetic Design is describing plant stem cell technology, citing Eric Perrier of LVMH as saying plant stem cell extracts are "efficacy in a jar." The controversy surrounding stem cells has only fueled the interest of cosmetic scientists in search of ingredients that promise to reverse signs of aging. Paving the way, the incarnation of cosmetic plant stem cell technology applications was introduced to the market by Dior and Amatonkin with high-end products that activated endogenous adult stem cells.

What are stem cells though? They are biological cells found in all multicellular organisms, which can divide, through mitosis, and differentiate into diverse, specialized cell types. Each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function, i.e. a skin cell, muscle cell, red blood cell or a brain cell! Stem cells serve primarily as an internal repair system. These cells can essentially, divide without limit, to replenish other cells or migrate to damaged areas to repair tissue.

Code Number: 16587

INCI Name: Euterpe Oleracea Fruit Extract

INCI Status: Conforms

REACH Status: Compliant

CAS Number: 999999-99-4

EINCS Number: 310-127-6

Origin: Botanical

Processing:

GMO Free

No Ethoxylation

No Irradiation

No Sulphonation

Additives:

Preservatives: None

Antioxidants: None

Other additives: None

Solvents Used: N/A

Appearance: Clear to Slightly Hazy Liquid

Soluble/ Miscible: Water

Ecological Information:

100% Biodegradability

Microbial Count: <100 opg,
No Pathogens

Suggested Use Levels: 1.0 – 10.0%

Suggested Applications: Anti-aging, Anti-wrinkle, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism

Benefits of **Phyto-Biotics Acai®**:

- Stem Cell Technology
- Anti-Aging
- Skin and Hair Care Applications
- Antioxidant

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Cellular plasticity is a specific characteristic of stem cells. This is the cell's ability to move from an undifferentiated state to a specific cell type. Regardless of their source, be it plant or animal, all stem cells are defined by their plasticity. There are two types of plasticity; pluripotent, cells that can transform from a generic plant or animal cell into many different cell types, and totipotent, cells that can transform into any cell type. Meristemic cells are pluripotent cells found in plants, which, along with stem cells, have the ability to replicate beyond Hayflick's Limit.

Research shows that plant stem cells can slow skin aging by defending against extrinsic stress, keeping skin looking youthful, longer. This discovery opened the door in cosmetic stem cell research and provided technological advances in the cosmetic industry, as there are no ethical controversies over the use of plant stem cells in contrast to the use of embryonic stem cells.

Two approaches to stem cell technology are currently used: The stimulation of adult stem cell proliferation and the use of plant stem cells, or Meristematic cells. The use of meristematic cells is currently the most popular form of stem cell technology and typically uses non-differentiated cells from simple cell extracts. These traditional stem cell products provide no specific activity and cannot provide specific cosmetic benefits.

Phenolic, or secondary, metabolites are organic compounds that have no fundamental role in the maintenance of the life process of plants. However, these compounds are essential for the plant to interact with its environment, allowing for adaptation, defense and ultimately the ability to survive in less than ideal conditions. Examples of plants producing secondary metabolites can be found in a plant's floral scent and pigment that have evolved to attract pollinators, enhancing fertilization rates. Some plants are able to synthesize toxic chemicals to ward off pathogens, herbivores or to suppress the growth of neighboring plants. Meanwhile, chemicals found in fruits prevent spoiling and send signals in the form of color, aroma and flavor to animals that eat the fruit and help disperse the seeds.

Of particular interest, for the cosmetic use of secondary metabolites to create activity specific plant stem cells, are Acai palms. Native to the tropical Central and South American climates, Acai Palms grow from Belize to the south of Brazil and Peru, in flood plains and swamps. This extreme environment, including high temperatures, intense UV radiation and excessive exposure to water, has forced the Acai Palm to evolve, using secondary metabolites that are essential for the palm to interact with and survive in its environment. As one can imagine, the secondary metabolites produced by the Acai Palm are potent antioxidants, protectants and Reactive Oxygen Species (ROS) scavengers. The secondary metabolite, or phenolic metabolite, of interest from Acai is Ferulic Acid. Found in the plant's cell wall components, Ferulic Acid readily forms a resonance stabilized phenoxy radical, acting as a potent antioxidant and ROS scavenger. Implicated in DNA damage and the acceleration of cell aging, ROS and free radicals are two extrinsic sources of stress from which the skin needs protection.

The destruction of plants continues to pose a major threat to the plant species. Commercially, the medicinal and cosmetic use of plant secondary metabolites involves isolating these organic compounds through solvent extraction of the naturally grown, whole plant. With the possibility of extinction at any given time, the development of alternative and complimentary methods to whole plant extract for the production of these organic compounds, particularly for commercial purposes, is an issue of considerable socioeconomic importance.

By using cell cultures, industries can grow numerous plants, creating a sustainable source of plant extracts without disrupting the environment. In studies, secondary metabolites have been observed in much higher concentrations in cultured cells than in whole plants of the same species. Perhaps this is due to plant's ability to synthesize a remarkable variety of secondary metabolites in order to adjust their metabolic activities as a response to biotic and/or abiotic stress. Inducing secondary metabolites through biotic, pathogenic stress, specifically in the presence of bacteria, Active Concepts has found a way to provide a unique and active active stem cell product.

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BENEFITS

Phyto-Biotics Acai® capitalizes on the specific activity provided by Ferulic Acid to provide formulators with a plant-derived, stem cell ingredient capable of imparting anti-aging, antioxidant, anti-wrinkle, and protectant benefits while enhancing ATP synthesis and cellular metabolism. This novel approach gives formulators the distinct opportunity of creating activity-specific products by using an advanced stem cell technology.

EFFICACY

To demonstrate the improvements in Ferulic Acid content in Acai Palms co-cultured with *Leuconostoc sp.*, an efficacy assay was performed comparing it to un-inoculated controls. Genetically uniform, shoot-based clonal lines of Acai were isolated and co-cultured in 1 mg/ 1 benzylaminopurine in standard Murashige and Skoog medium with 3.0% sucrose and inoculated with *Leuconostoc sp.* for thirty (30) days. The controls consisted of seven (7), un-inoculated clonal lines of Acai. After thirty (30) days, Ferulic Acid was extracted from 50 mg of the plant tissues with 2ml of 50% (v/v) methanol for one (1) hour at 55°C. After cooling to room temperature, 1 ml of extract was diluted by adding 3 ml of 50.0% (v/v) methanol. The absorbance was measured at 333 nm with a Spectronic.RTM.Genesys.TIM.5 spectrophotometer. The improvements in Ferulic Acid content in the Acai Palms co-cultured with *Leuconostoc sp.* in comparison to the control are clearly indicated in the following graph:

Improvements in Ferulic Acid Content in Acai Co-Cultured with *Leuconostoc sp.*

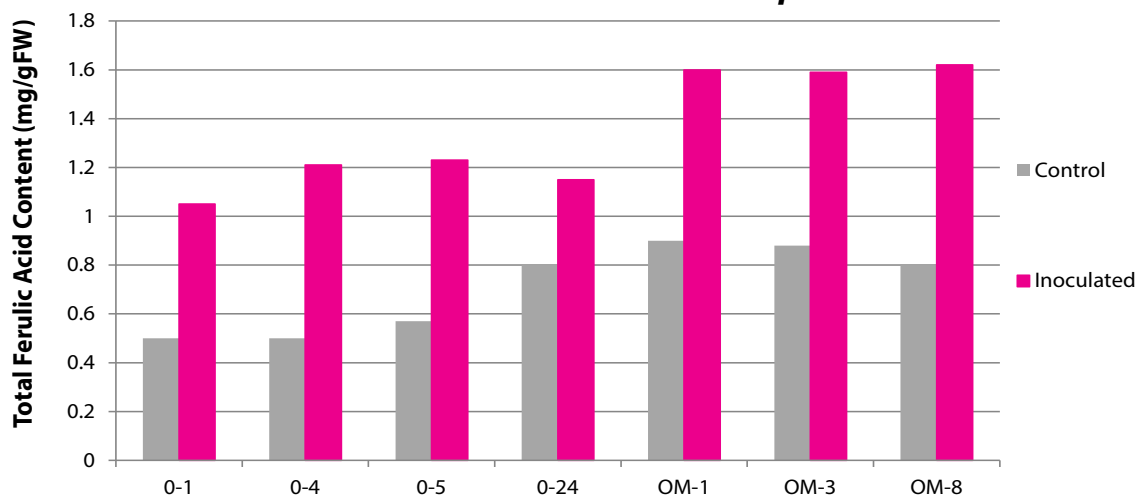


Figure 1. Compared to the control Ferulic acid content increases when Acai is co-cultured with *Leuconostoc sp.*

UV absorption by Ferulic Acid catalyzes stable phenoxy radical formation, and thereby, potentiates its ability to terminate free radical chain reactions. The antioxidant benefits of Ferulic Acid are more potent than those obtained from Vanillic, Coumaric, or Cinnamic Acid treatments, which are commonly associated with strong antioxidant protection. To demonstrate Ferulic Acid’s ability to provide antioxidant benefits in cosmetic formulations, a study was conducted to test the increase in photo-protection and stability when using Ferulic Acid, Vitamin C, and Vitamin E. Skin was pretreated with 75, 150, and 250 mL of 15.0% Vitamin C, 1.0% Vitamin E, and 0.5% Ferulic Acid and then irradiated with solar-stimulated radiation at a minimal erythema dose (MED) of 1 to 5 at 1 MED intervals. The skin was evaluated one day later with: Colorimeter measurements of photo-protection by Vitamins C, E and Ferulic Acid (n=3); and Sunburn cell measurements of photo-protection by Vitamins C, E, and Ferulic Acid (n=3).

In Figure 2 (below), research surrounding the effect of dosage on photoprotection provided by Vitamins C and E in addition to Ferulic Acid are being evaluated. The purpose of this study was to evaluate the effect of dosage on skin protection using known antioxidant solutions. The skin was pretreated with 75, 150 and 250mL of 15.0% Vitamin C, 1.0% Vitamin E and 0.5% Ferulic Acid. The skin was then irradiated with solar stimulated radiation at selected minimal erythma dose. The results demonstrate that sunburn protectino is dose dependent.

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Effect of Dosage on Skin Photo Protection

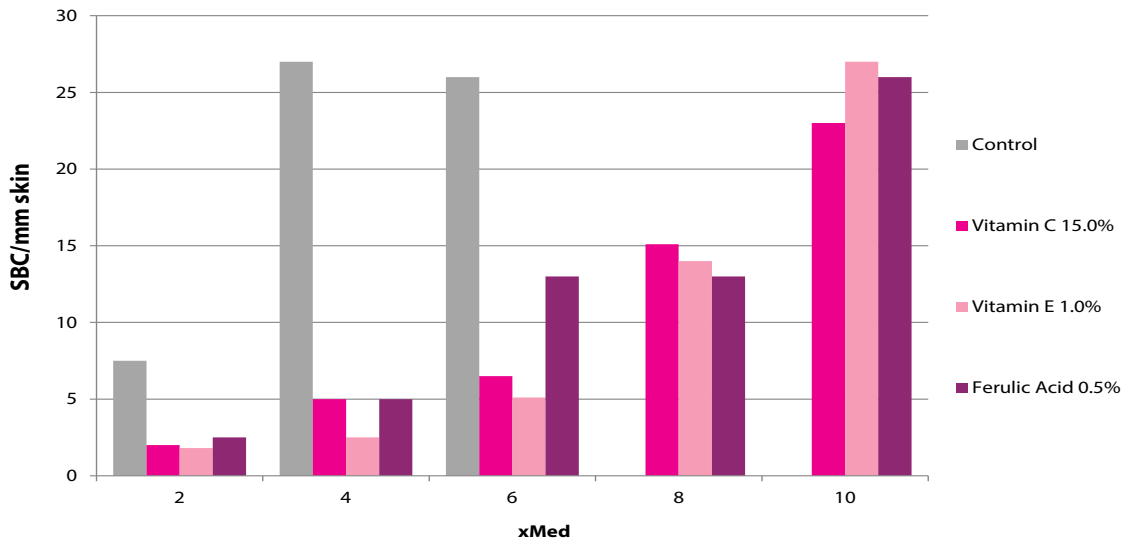


Figure 2. Effects of dosage on photo-protection when using Vitamins C, E and Ferulic Acid.

Additionally, the effect of Ferulic Acid on Photo-Protection and Stability was evaluated. Researchers compared the differences between a (control) vs .(vehicle) vs. (Ferulic Acid) vs. (Vitamin C + Vitamin E) vs (Ferulic Acid + Vitamin C+ Vitamin E). The skin was pretreated with a vehicle, 0.5% Ferulic Acid, 15.0% Vitamin C and 1.0% Vitamin E, 15.0% Vitamin C and 1.0% Vitamin E and 0.5% Ferulic Acid. The skin was then irradiated with solar-stimulated radiation 2x - 10x the minimal erythema dose. The skin patches were evaluated one day later.

(Reference: Fu-Hsiung L., et al. Ferulic Acid Stabilizes a Solution of Vitamins C and E and Doubles its Photoprotection of Skin. Journal of Investigative Dermatology (2005) 125, 826-832; doi: 10.1111/j.0022-202X.2005.23768x)

Colorimeter Measurements of Photoprotection by Antioxidant Solutions

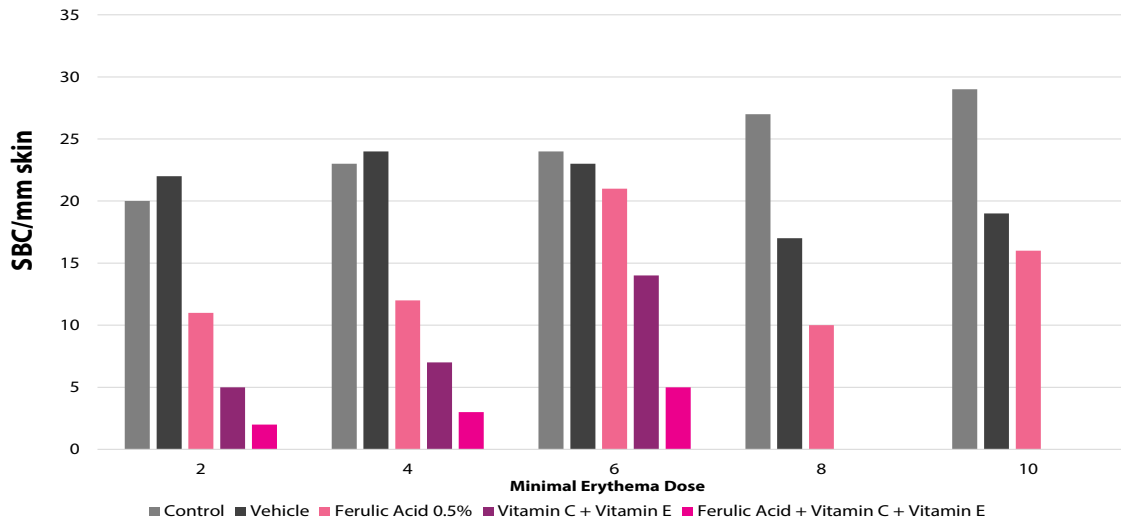


Figure 3. Colorimeter measurements of photo-protection by antioxidant solutions

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Visual Erythema + Antioxidant Photoprotection by Antioxidant Solutions

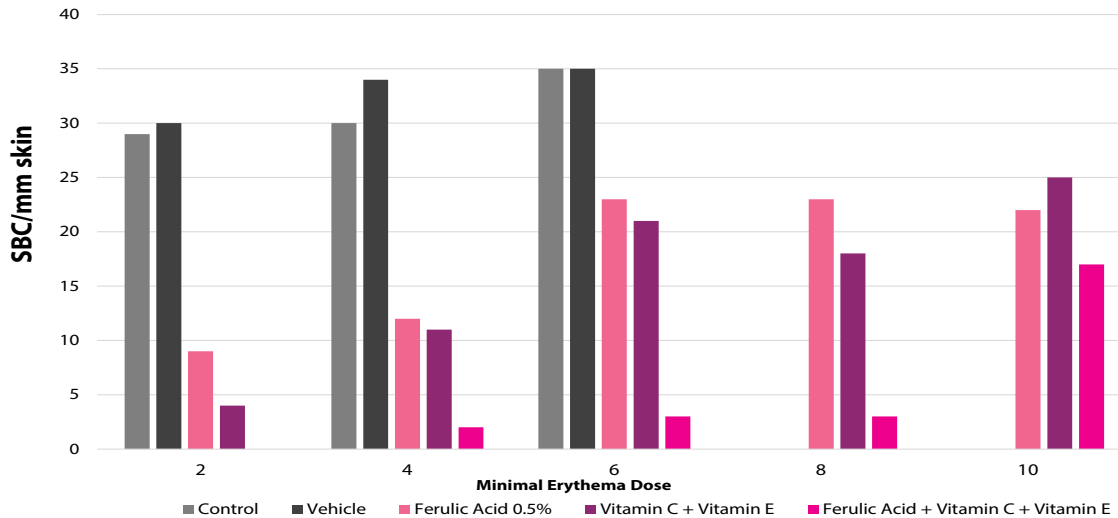


Figure 4. Effects of dosage on photo-protection when using Vitamins C, E and Ferulic Acid..

An Oxygen Radical Absorbance Capacity Assay was conducted in-house to measure the antioxidant activity of **Phyto-Biotics Acai®** co-cultured with *Leuconostoc sp.* standardized for 5.0% Ferulic Acid was compared with a Trolox Control (Vitamin E derivative and antioxidant standard in the cosmetic market).

ORAC Assay

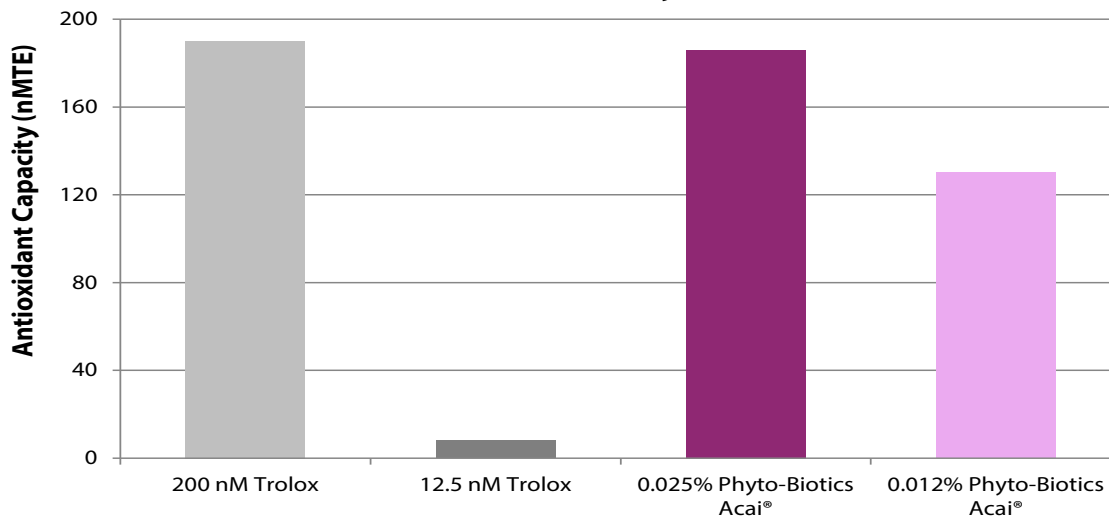


Figure 5. The results indicated that **Phyto-Biotics Acai®** provides comparable antioxidant activity to Trolox and is suitable for use as a protectant and cosmetic formulations.

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-kB) 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.

An Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in **Phyto-Biotics Acai®**. Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete DMEM.

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1.0%, 0.1%, and 0.01% concentrations of **Phyto-Biotics Acai®**, standardized for 5.0% Ferulic Acid, were added to complete DMEM containing 1Mg/mL LPS and incubated with fibroblasts for 24 hours. Complete media containing 1Mg/mL LPS was used as the positive control, and complete DMEM was used as a negative control. IL-6 production percent decrease is calculated by using the following formula:

$$\frac{(\text{Positive Control Average Concentration} - \text{Sample Average Concentration})}{(\text{Positive Control Average Concentration})} \times 100$$

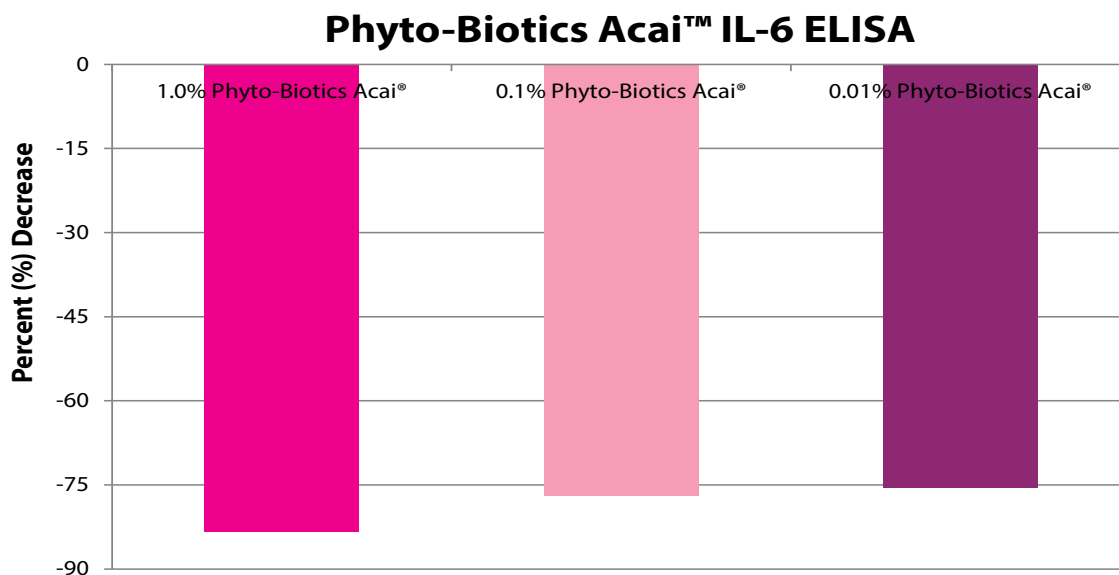


Figure 6. Decrease in IL-6 production indicates a reduction of an inflammatory environment.

This decrease in IL-6 production when using **Phyto-Biotics Acai®** indicates a reduced inflammatory environments, which could decrease the signs of aging and reduce the formation of fine lines and wrinkles. From this information, it can be ascertained that **Phyto-Biotics Acai®** is suitable for cosmetic applications designed to provide soothing and anti-aging benefits.

Furthermore, a Cellular Viability Assay was conducted to quantitatively measure 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 in ATP levels indicates cytotoxicity and decreased cell function, while an increase in ATP levels indicates healthy cells. The cellular viability assay conducted was to assess the ability of **Phyto-Biotics Acai®** to increase cellular metabolic activity in cultured dermal fibroblasts. 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®** concentration 0.01% in complete DMEM and incubated with fibroblasts for 24 hours. Ten microliters of viability reagent was added to 90ML of cell culture media in culture wells. The resulting data obtained from this study demonstrated that **Phyto-Biotics Acai®** at a concentration of 0.01% is able to increase cellular metabolism compared to the control.

As shown in Figure 7, **Phyto-Biotics Acai®** exhibited positive results by increasing cellular metabolism. This data indicates that **Phyto-Biotics Acai®** is ideal for cosmetic applications designed to increase cell viability and metabolism.

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Cellular Metabolism of Phyto-Biotics Acai®

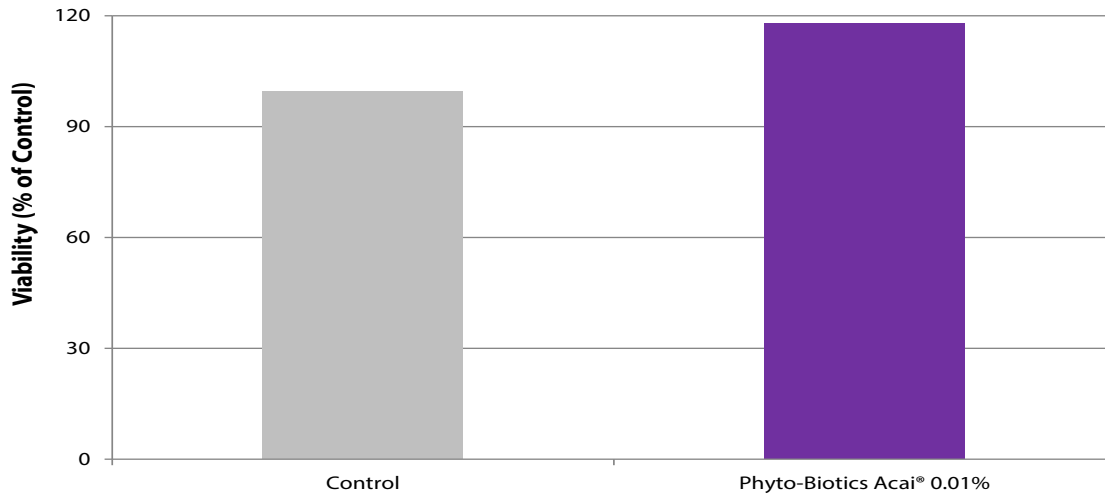


Figure 7. Cellular Metabolism of **Phyto-Biotics Acai®** treated in fibroblasts expressed in terms of percent of control.

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. 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. Results indicate that this material is capable of significantly increasing moisturization compared to the control.

Average Moisturization

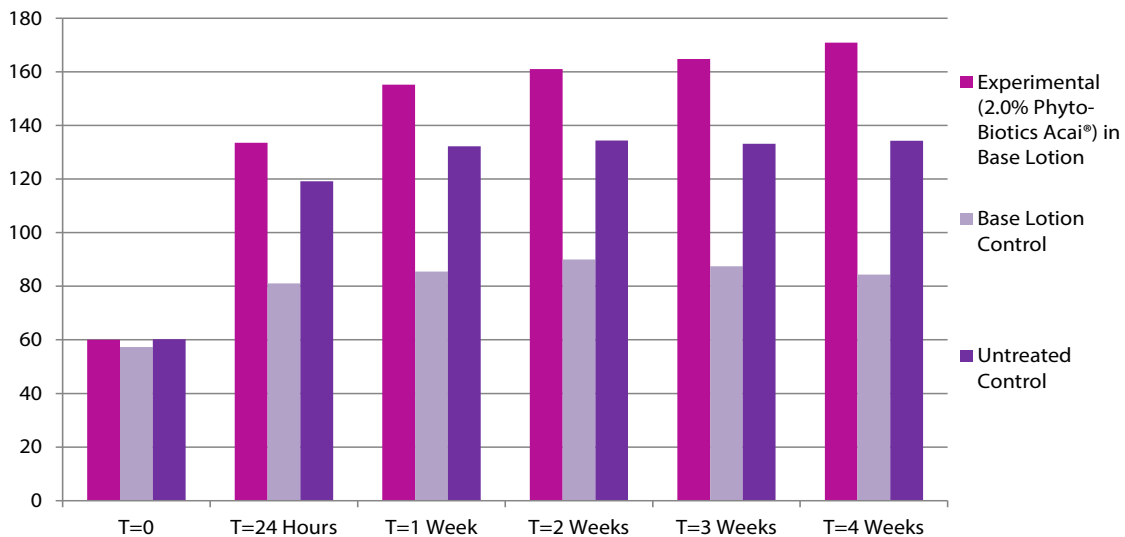


Figure 8. Average moisturization levels measured at each test site.

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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Comparative Moisturization

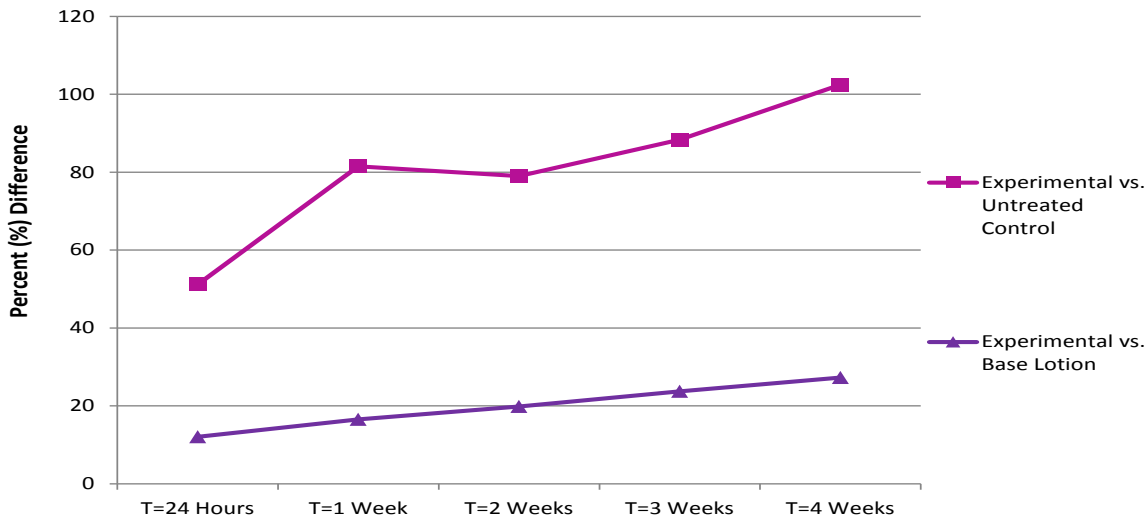


Figure 9. Comparative moisturization analysis between each test site.

Moisture Regression Experimental Treatment vs. Untreated

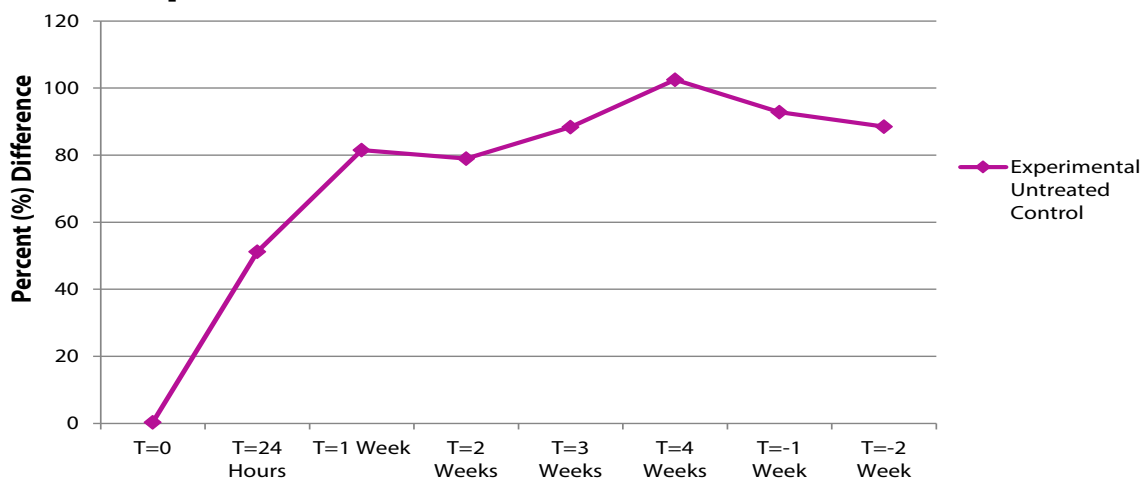


Figure 10. Comparative analysis of moisture regression over time.

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.

With a continued demand for products that embody plant stem cell technologies, it is imperative for products to provide specific activity for brand differentiation. **Phyto-Biotics Acai®** is your answer to an innovative stem cell approach, which gives formulations the distinct opportunity to create activity-specific products to propel past their competition.

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