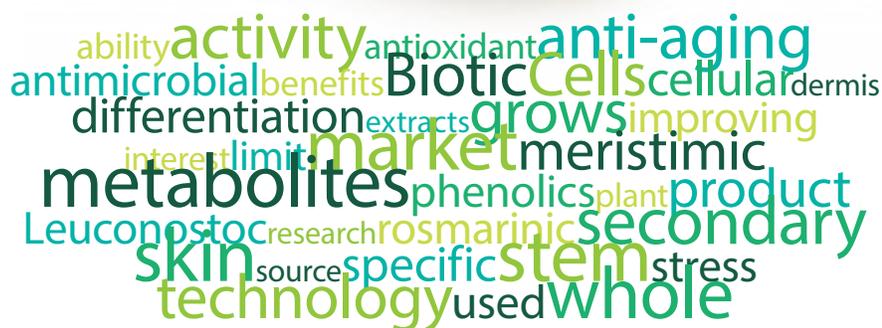


# Phyto-Biotics Perilla®



## BACKGROUND

The demand for stem cell technologies has grown exponentially in the last decade. Trending specifically in the cosmetic market, current stem cell technologies utilize Meristematic cells, non-differentiating plant stem cells from simple cell extracts with no specific activity. To propel beyond the current offerings on the market, Active Concepts has chosen to use biotic stress, embodied by the sustainable practice of co-culturing of plant stem cells with *Leuconostoc sp.*, to promote the formation of secondary metabolites. This novel approach induces cellular differentiation and provides formulators with the ability to finely customize formulations, specify activity and skin benefits while offering brand distinction. **Phyto-Biotics Perilla®** capitalizes on specific activity of the phenolic metabolite, Rosmarinic Acid, produced by *Perilla frutescens*, or Chinese Basil, to provide the cosmetic market with a plant-derived, stem cell ingredient designed to impart antioxidant, protectant, antimicrobial and anti-aging benefits while enhancing cellular metabolism and improving radical stability.

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.

**Code Number: 40600**

**INCI Name:** Perilla Frutescens Extract

**INCI Status:** Approved

**REACH Status:** Complies

**CAS Number:** 90082-61-4

**EINECS Number:** 290-151-0

**Origin:** Botanical

**Processing:**

GMO Free

No Ethoxylation

No Irradiation

No Sulphonation

**Additives:**

Preservatives: None

Antioxidants: None

Other additives: None

**Solvents Used:** Water

**Appearance:** Clear to Slightly Hazy Liquid

**Soluble/ Miscible:** Water Soluble

**Ecological Information:**

86.25% Biodegradability

**Microbial Count:** <100 CFU/g, No Pathogens

**Suggested Use Levels:** 1.00 - 10.00%

**Suggested Applications:**

Anti-Aging, Soothing, Antioxidant

Benefits of **Phyto-Biotics Perilla®**:

- Increases Cellular Metabolism
- ATP Synthesis
- Stem Cell Technology

# Phyto-Biotics Perilla®

## SCIENCE

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.

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. Currently, there are two approaches to stem cells: The stimulation of adult stem cell proliferation and the use of plant stem cells.

There are stem cells located in an area of the dermis referred to as the follicular bulge. These stem cells are capable of differentiating into keratinocytes and epithelial cells. However, our skin contains cells that do more than just produce structural proteins and pigments. The dermis also exhibits neuronal and immunological activity. Unfortunately, enhancing the proliferation of stem cells found at the follicular bulge will not improve the other activities produced by the dermis.

The use of plant stem cells, or Meristematic cells, is currently the most popular form of stem cell technology. This technology, which uses non-differentiated cells from simple cell extracts, provides no specific activity and therefore cannot be used to provide specific cosmetic benefits. To separate from the competition and project ourselves into the future, Active Concepts conceptualized the idea of using biotic stress to induce plant secondary metabolites, or differentiation of plant stem cells.

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.



# Phyto-Biotics Perilla®

The use of *Perilla frutescens*, or Chinese Basil, spread throughout Asia sometime in the remote antiquity. The fresh leaves of this herb are used in Asian cuisine, particularly salads, soups and as garnishes for entrees such as sushi. A popular medicinal treatment, particularly in China and Japan, the leaves of *Perilla frutescens* are used to treat asthmas and coughs, while the seeds, a rich source of Omega-3 alpha-linoleic acid, are said to support a healthy immune system. This herbal plant grows best in moist, humid, organically rich soil, however, its resistance to heat and drought have allowed *Perilla frutescens* to flourish even in the most desolate conditions. The secondary metabolites produced during times of stress have supplied *Perilla frutescens* with potent antioxidant and soothing properties, helping lessen systemic damage caused by free radicals and resist impending inflammation.

Of particular interest, the phenolic compound, Rosmarinic Acid has demonstrated antimicrobial and antioxidant properties. Due to genetic heterogeneity, or the breeding character being influenced by natural cross-pollination, the content of this phenolic metabolite is highly variable. By co-culturing *Perilla frutescens* with *Leuconostoc sp.*, with Active Concepts has discovered a means of preventing hyperhydricity to increase the total Rosmarinic Acid content in Chinese Basil.

Rosmarinic Acid is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid. It possess four phenolic hydrogens that contribute to its ability to control free radical oxidation. Additionally, it contains two catechol (1,2-dihydroxybenzene) rings which gives Rosmarinic Acid a quality of polarity. This means that the phenolic compound can form intermolecular hydrogen bonds between the free hydrogen of its hydroxyl and of its phenoxy radical, significantly improving its radical stability.

Rosmarinic Acid strongly inhibits 5-lipoxygenase products, 5-hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE) and Leukotriene A4 (LTB4) at concentrations of 10<sup>-5</sup> and 10<sup>-3</sup>M. In addition to Rosmarinic Acid, inhibition of 5-HETE and LTB4 were exhibited to a degree in the subsequent, following order: Caffeic Acid, Caffeoyltartaric Acid, Caffeoylmalic Acid, and Chlorogenic Acid. Furthermore, an added benefit of Rosmarinic Acid is that it provides antibacterial activity, specifically against *Bacillus subtilis*, *Micrococcus luteus*, and *Escherichia coli*. These aforementioned benefits imply that Rosmarinic Acid has potential as a therapeutic treatment for allergic reactions or inflammation and an antimicrobial.

## BENEFITS

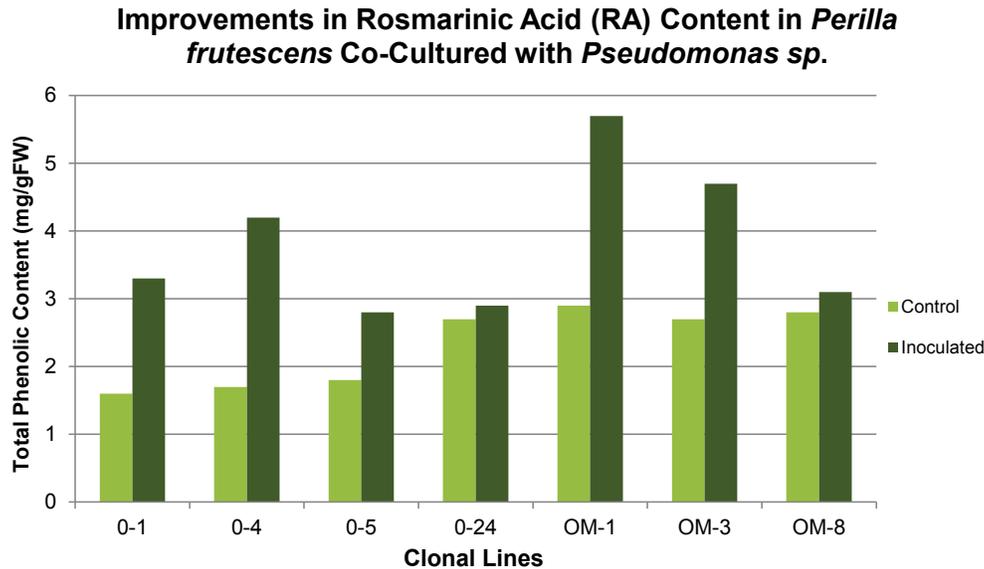
The destruction of plants continues to pose a major threat to the plant species. Commercially, the medicinal and cosmetic use of plant secondary metabolites involves isolating these organic compounds through solvent extraction of the naturally grown, whole plant. With the possibility of extinction at 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.

As previously mentioned, using solvent extraction from a naturally grown, a whole plant is environmentally damaging. In order to sustainably source the necessary phenolic metabolite, Rosmarinic Acid, Active Concepts grows *Perilla frutescens* in cell culture. Using biotic stress, specifically pathogenic stress via *Leuconostoc sp.*, our formulators created **Phyto-Biotics Perilla®** by inducing the production of the phenolic compound, Rosmarinic Acid, to create a plant stem cell product ideal for anti-aging, antimicrobial and soothing cosmetic applications.

## EFFICACY

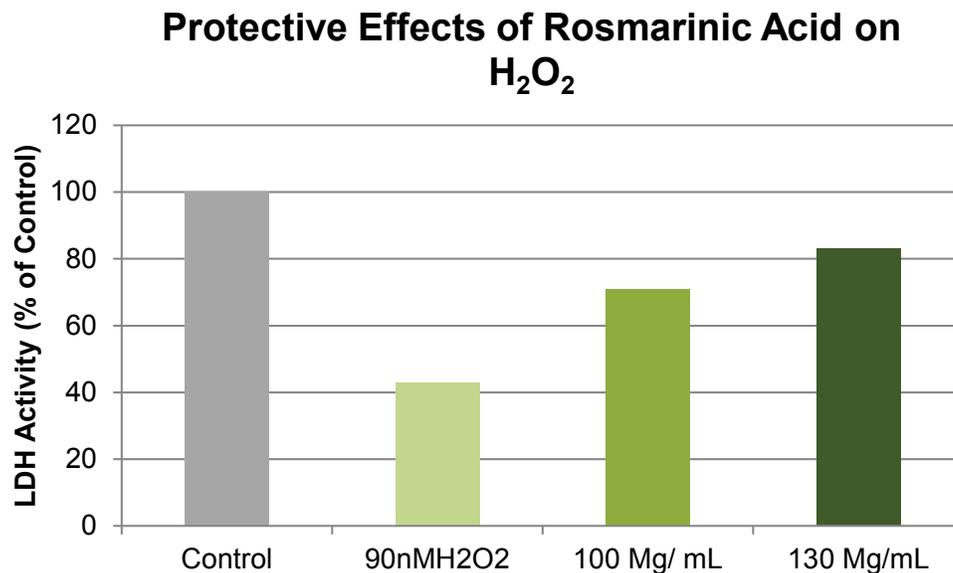
Active Concepts drew inspiration for **Phyto-Biotics® Perilla** from previous research demonstrating the improvements in Rosmarinic Acid content in *Perilla frutescens* co-cultured with *Pseudomonas sp.* compared to un-inoculated controls. Genetically uniform, shoot-based clonal lines of *Perilla frutescens* were isolated and co-cultured in 1 mg/ 1 benzylaminopurine in standard Murashige and Skoog medium with 3.0% sucrose and inoculated with *Pseudomonas sp.* for thirty (30) days. The controls consisted of seven (7), un-inoculated clonal lines of *Perilla frutescens*. After thirty (30) days, Rosmarinic 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 Rosmarinic Acid content in the *Perilla frutescens* herbal plants co-cultured with *Pseudomonas sp.* in comparison to the control are indicated in the following graph.<sup>1</sup>

# Phyto-Biotics Perilla®



**Figure 1.** Improvements in Rosmarinic Acid in *Perilla frutescens* co-cultured with *Pseudomonas sp.*<sup>1</sup>

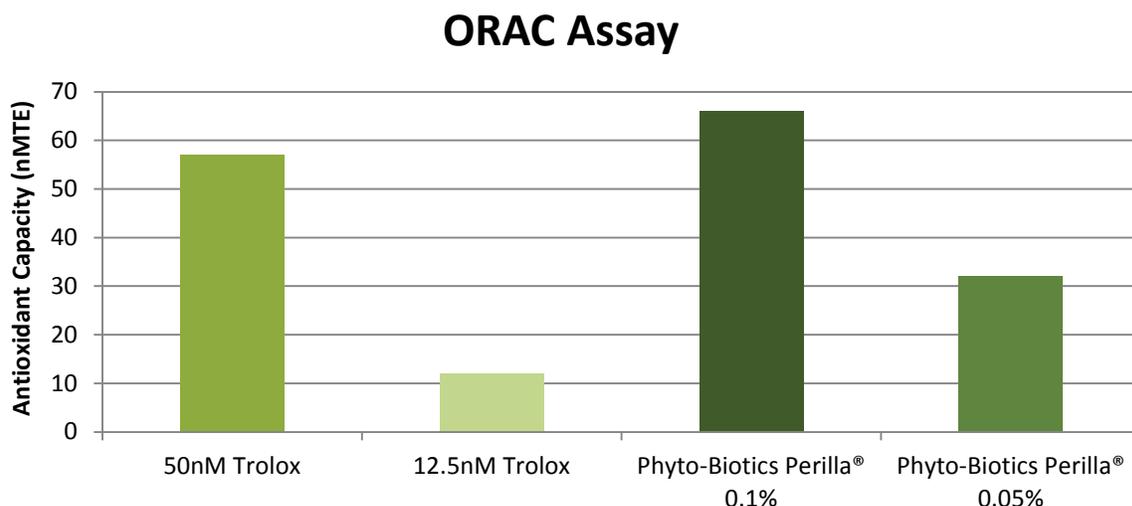
To demonstrate the protective benefits of Rosmarinic Acid, a test was conducted on H<sub>2</sub>O<sub>2</sub>. To examine the effects of Rosmarinic Acid on H<sub>2</sub>O<sub>2</sub> the cultured solutions containing Rosmarinic Acid, at each concentration from 100 to 130 nM, were incubated for two (2) hours.



**Figure 2.** Increase in LDH activity and the protective effects of Rosmarinic Acid.

An Oxygen Radical Absorbance Capacity Assay was conducted in-house to measure the antioxidant activity of **Phyto-Biotics Perilla®**. **Phyto-Biotics Perilla®** co-cultured with *Leuconostoc sp.* standardized for Rosmarinic Acid was compared with a Trolox Control (Vitamin E analogue and antioxidant standard for free radical

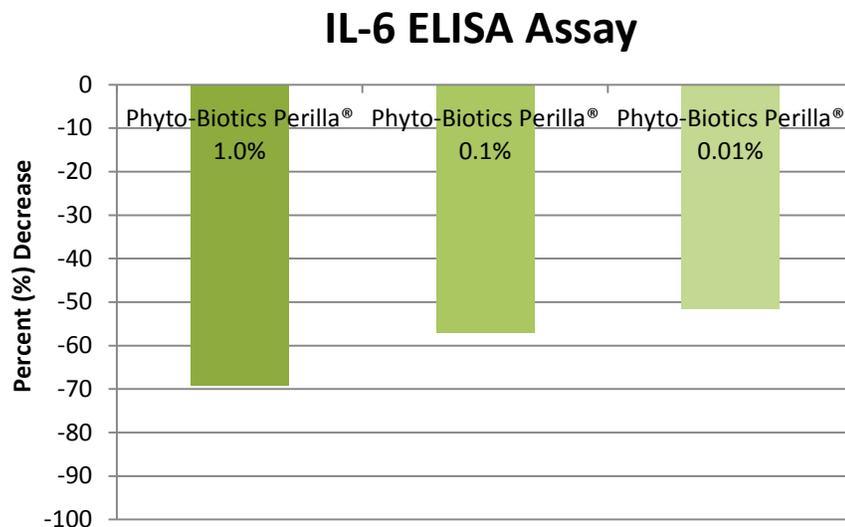
# Phyto-Biotics Perilla®



**Figure 3.** The results indicated that **Phyto-Biotics Perilla®** provides comparable antioxidant activity to Trolox and is suitable for use as a protectant and antioxidant in cosmetic formulations.

The results indicated that **Phyto-Biotics Perilla®** exhibited strong antioxidant activity at 0.1%. As demonstrated in the graph, Phyto-Biotics Perilla® has antioxidant activity comparable to that of Trolox. Due to its potent antioxidant activity, Phyto-Biotics Perilla® can be used in cosmetic applications to impart anti-aging benefits compliments of the product’s potent antioxidant properties.

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 Perilla®**. Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1.0%, 0.1%, and 0.01% concentrations of **Phyto-Biotics Perilla®**, standardized for Rosmarinic 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.



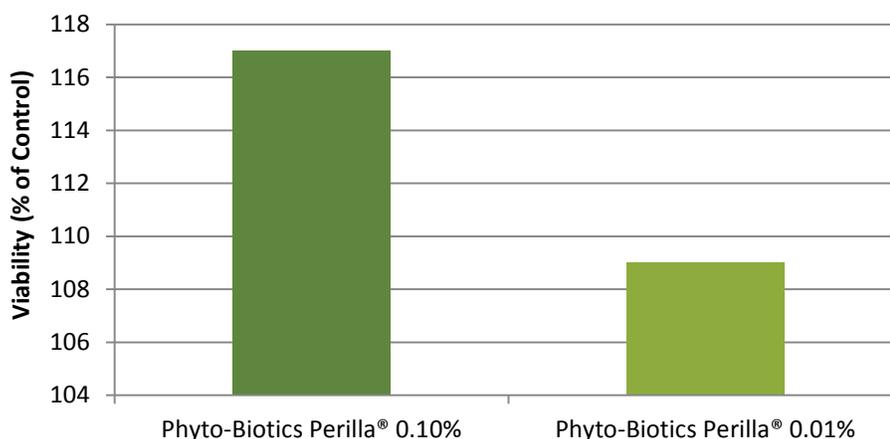
**Figure 4.** Decrease in IL-6 production when using **Phyto-Biotics Perilla®** indicates a reduced inflammatory environments.

# Phyto-Biotics Perilla®

This decrease in IL-6 production when using **Phyto-Biotics Perilla®** indicates a reduced inflammatory environment, 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 Perilla®** 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 Perilla®**, standardized for Rosmarinic Acid, 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 Perilla®** concentrations on 0.1% and 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 Perilla®** at all concentrations is able to increase cellular metabolism compared to the control.

## Cellular Metabolism



**Figure 5.** Cellular Metabolism of **Phyto-Biotics Perilla®** treated in fibroblasts expressed in terms of percent control.

As shown in Figure 5, **Phyto-Biotics Perilla®** exhibited positive results by increasing cellular metabolism. This data indicates that **Phyto-Biotics Perilla®** is ideal for cosmetic applications designed to increase cell viability and metabolism.

With the continued demand for plant stem cell technologies, it is imperative for products to provide specific activity for brand differentiation. Propelling past the competition, Active Concepts created **Phyto-Biotics Perilla®**, created using biotic stress from *Leuconostoc sp.* and the sustainable practice of co-culturing plant stem cells to induce the secondary, phenolic metabolite, Rosmarinic Acid. **Phyto-Biotics Perilla®** capitalizes on the specific activity provided by Rosmarinic Acid to provide formulators with a plant-derived, stem cell ingredient capable of imparting anti-aging, antioxidant, antimicrobial, 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 and brand distinction using advanced stem cell technology.

# Phyto-Biotics Perilla®

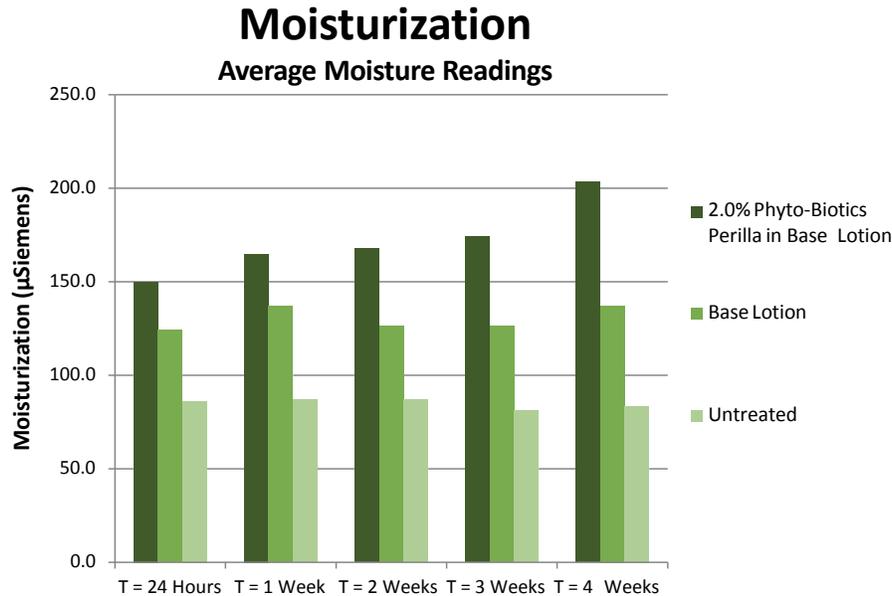


Figure 6. Average increase in moisturization per test site.

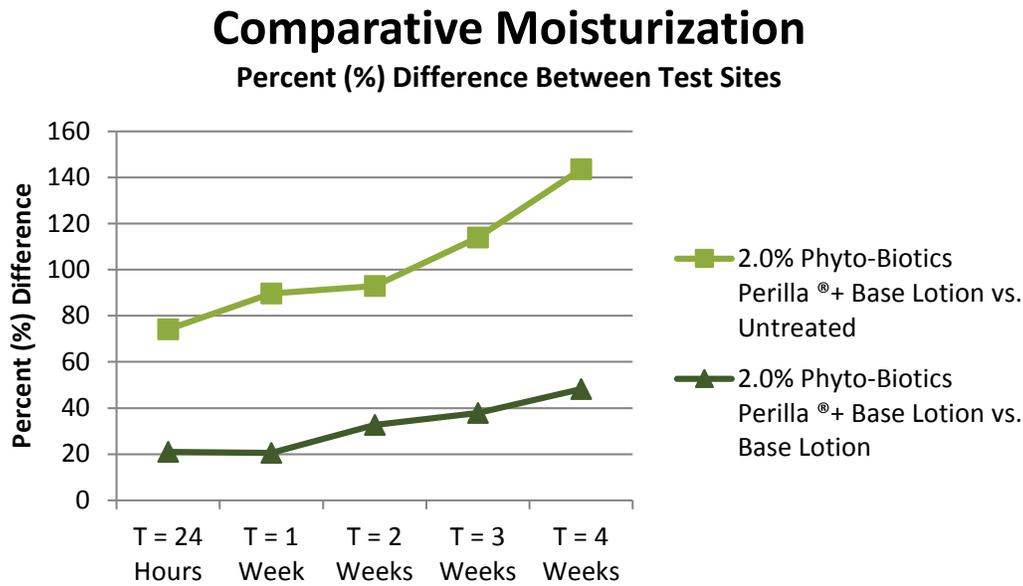


Figure 7. Percent difference in moisturization between two test sites over four weeks.

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.

# Phyto-Biotics Perilla®

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 readings were taken one week after the applications of both test materials ceased.

**Phyto-Biotics Perilla®** was designed to provide moisturization 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.

## References:

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- 2) Osakabe N, et al. Rosmarinic acid inhibits epidermal inflammatory responses: anticarcinogenic effects of Perilla frutescens extract in the murine two-stage skin model. Carcinogenesis. 2004 Apr; 25(4): 549-57. Epub 2004 Jan 16. Health and Bioscience Laboratories, Meiji Seika Kaisha Ltd, 5-3-1, Chiyoda Sakado 350-0289.
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