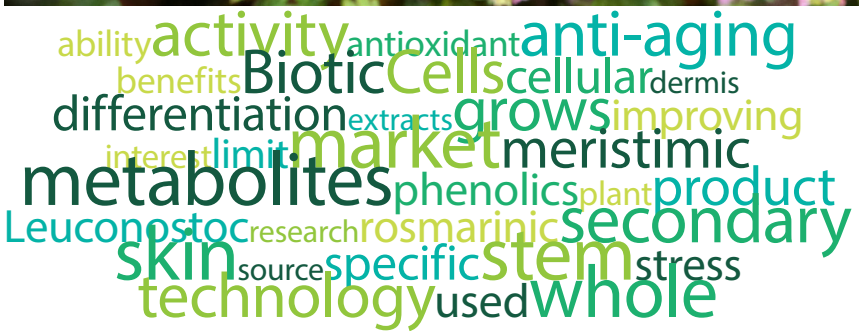
Phyto-Biotics Perilla®



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, Moisturizing, Antioxidant, Anti-inflammatory

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, 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.

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

- Increases Cellular Metabolism
- Promote Collagen Synthesis
- Stem Cell Technology

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SCIENCE

What are stem cells? 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 what is known as 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 product 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.

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.*, Active Concepts has discovered a means of preventing hyperhydricity to increase the total Rosmarinic Acid content in Chinese Basil.

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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 phenoxyl 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 10-5 and 10-3M. In addition to Rosmarinc 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 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 *Leuconostoc 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 *Leuconostoc sp.* for thirty (30) days. The controls consisted of seven (7), un-inoculated clonal lines of *Perilla frutescens.* After thirty (30) days, Rosmarinc 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 *Leuconostoc sp.* in comparison to the control are indicated in the following graph.[1]

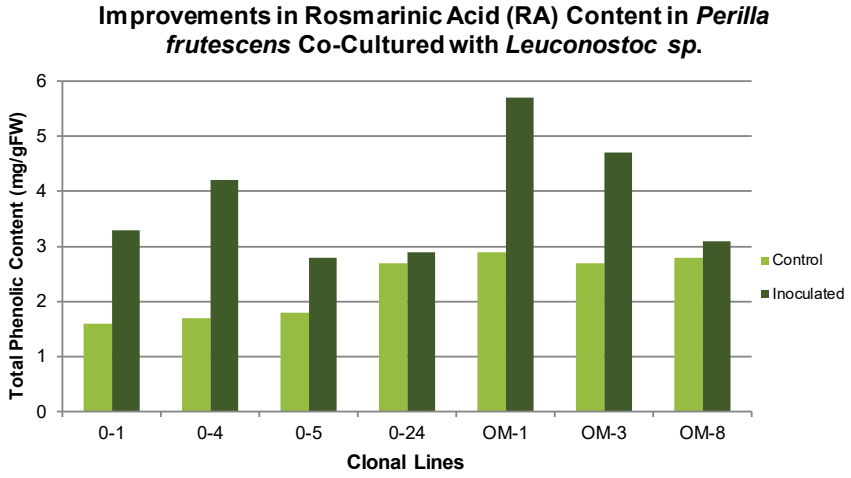

Figure 1. Improvements in Rosmarinic Acid in *Perilla frutescens* co-cultured with *Leuconostoc sp.*[1]

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An *in-vitro* Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **Phyto-Biotics Perilla®**. As shown in Figure 2, **Phyto-Biotics Perilla®** exhibited antioxidant activity comparable to Trolox®. The antioxidant capacity of **Phyto-Biotics Perilla®** increased as the concentration increased. As a result we can assure that its ability to minimize oxidative stress is dose dependent and that **Phyto-Biotics Perilla®** is capable of providing antioxidant properties.

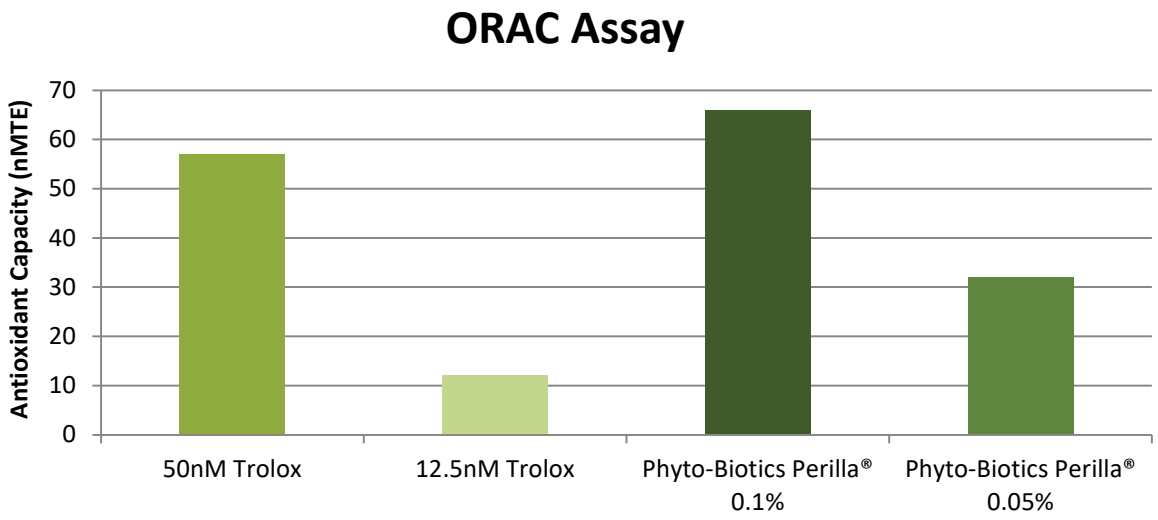

Figure 2. Antioxidant capacities.

An *in-vitro*, interleukin-6 ELISA assay was conducted to assess the changes in IL-6 levels in cultured human dermal fibroblasts treated with**Phyto-Biotics Perilla®**. 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 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. As shown in Figure 3, results indicate **Phyto-Biotics Perilla®** exhibited anti-inflammatory effects on LPS-treated fibroblasts utilizing various concentrations of **Phyto-Biotics Perilla®** including 1%, 0.1%, 0.01%. 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. This study indicates, at normal use concentrations, **Phyto-Biotics Perilla®** enhances soothing and anti-aging properties.

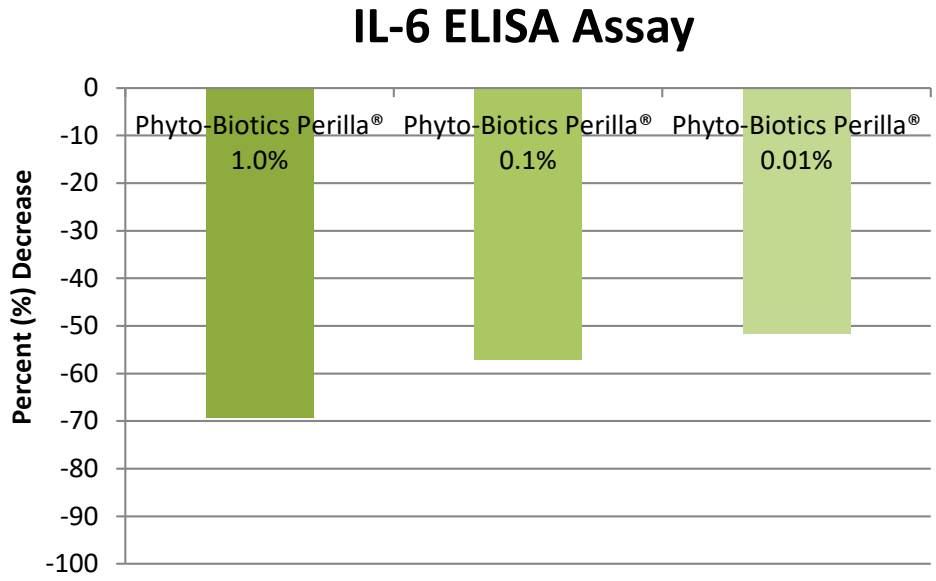

Figure 3. Anti-inflammatory capacity of **Phyto-Biotics Perilla®**.

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As evidenced in a 4 week *in-vivo* efficacy study of **Phyto-Biotics Perilla®** on skin, moisture levels were improved by 74.2% after 24 hours and by 143.6% after 4 weeks when compared to the untreated control. Comparisons of the base lotion to the Experimental Lotion containing 2.0% **Phyto-Biotics Perilla®** demonstrate the experimental material moisturized the skin 21.0% better after 24 hours. After four weeks the base lotion containing 2.0% **Phyto-Biotics Perilla®** moisturized skin 48.3% better than the base lotion alone. Results indicate that **Phyto-Biotics Perilla®** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion. Furthermore, when examining the moisture levels on the skin after application of test materials stopped, it was determined that **Phyto-Biotics Perilla®** is capable of sustaining increased skin moisturization when compared to the skin site that remained untreated through the duration of the study. **Phyto-Biotics Perilla®** demonstrates the ability to provide moisturization benefits to the skin in personal care applications.

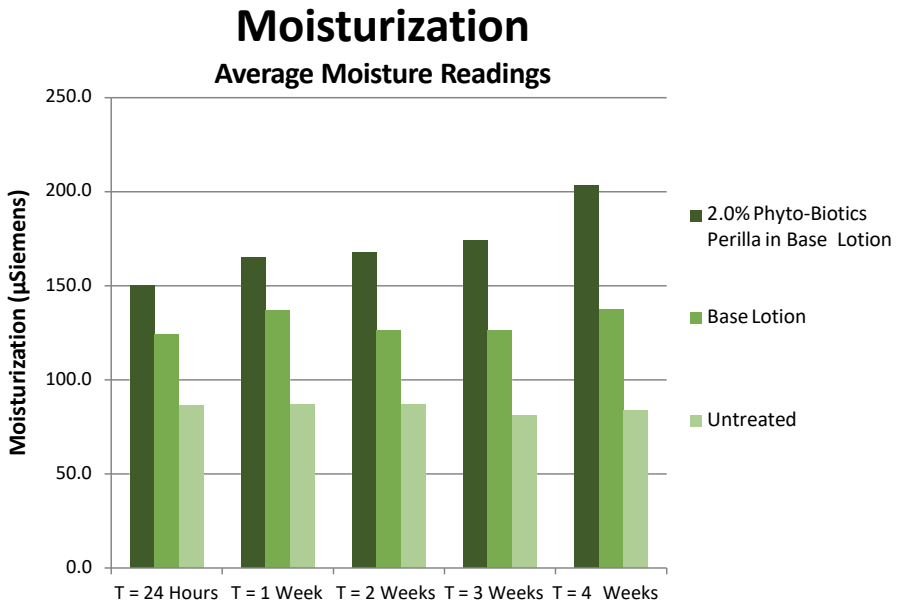

Figure 4. Average increase in moisturization per test site.

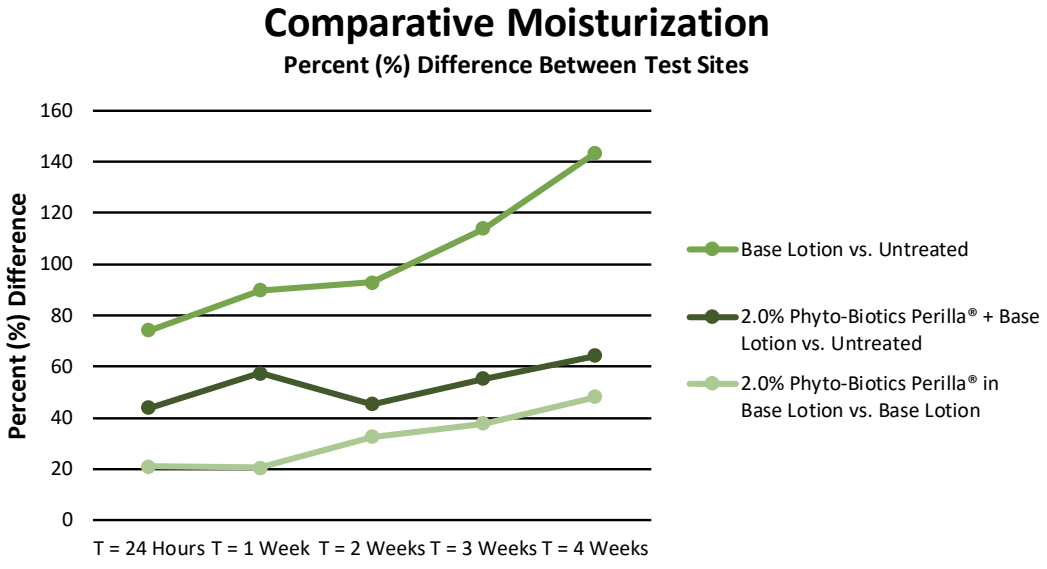

Figure 5. Percent difference in moisturization between test sites over four weeks.

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A *in-vitro* Sirius Red/Fast Green Assay was performed to assess the changes in collagen synthesis by **Phyto-Biotics Perilla®** treated cultured human dermal fibroblasts. Sirius Red is a unique dye that binds specifically to the helical structure of types I through V collagen, while Fast Green binds to non-collagenous proteins. These two dyes work in conjunction to provide a semi-quantitative method of determining amounts of collagen and non-collagenous proteins in a sample. After staining a sample the dyes are easily extracted and have optical density (OD) absorptions at 540nm (Sirius Red) and 605nm (Fast Green). Collagen concentrations are calculated through equations with OD values. Figure 6 demonstrates the collagen synthesis activity of **Phyto-Biotics Perilla.®**

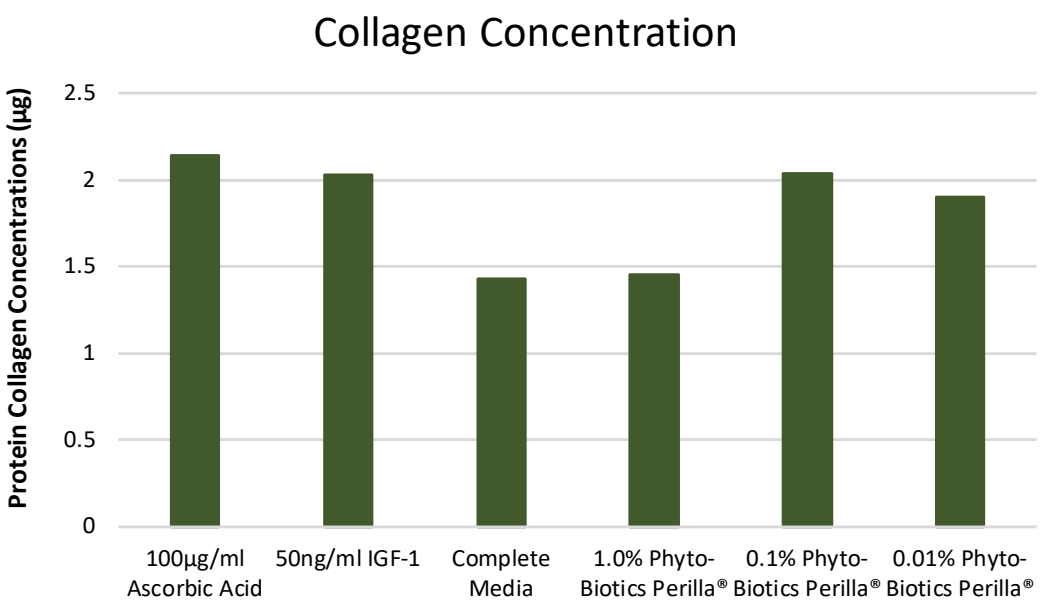

Figure 6. Comparative collagen concentration.

References:

1) Makino, T., et al. Anti-allergic effect of Perilla frutescens and its active constituents. Phutother Res. 2003 Mar;17(3):240-3. Department of Kampo Medicinal Sciences, Hokkaido College of Pharmacy, Katsuraoka, Otaru, Japan.
2) Osakable 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.
3) Pugliese, Michael Q., BS, L.E. Stem Cells: What and Why. Dermascope: The Publication of Aesthetics and Spa Therapy. Michael Q. Pugliese, BS, L.E. (2012).

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