

Phytofuse Rejuvenate®



sustainable powerful superfood
functional active ingredient with elegant feel
anti-inflammatory
film-former, moisturizing, protectant
soothing, wound healing

BACKGROUND

Salvia hispanica, commonly known as chia, is considered an ancient grain that has been around for centuries, but its extraordinary nutritional properties have led to a recent resurgence in the dietary supplement and food industries. *Salvia hispanica* is a desert plant native to central and southern Mexico and Guatemala that produces several small white and black seeds. The remarkable nutritional profile of these seeds surpasses that of any other superfood. *Salvia hispanica* seeds are a natural source of antioxidants that protect the skin from free radical damage. The seed also has a significant amount of protein and dietary fiber. With unprecedented hydrophilic properties, *Salvia hispanica* seeds produce a unique protective mucilage. When the seed is placed in water, it exudes a mucilaginous polysaccharide with the potential for use in innovative cosmetic and personal care applications. Active Concepts has sustainably isolated these natural mucilaginous polysaccharides to create **Phytofuse Rejuvenate®**, the next iteration of the Active Concepts' **Phytofuse** line. **Phytofuse Rejuvenate®** is capable of delivering exceptional wound healing, anti-inflammatory, film forming, and anti-irritant benefits as well as improving sensorial properties of any formulation.

"Chia" is the ancient Mayan word for "strength". Over time, skin loses strength and elasticity, resulting in fine lines and visible signs of aging. **Phytofuse Rejuvenate®** harnesses the therapeutic properties of *Salvia hispanica* seed mucilage to naturally strengthen and revitalize the skin. The Mayans and Aztecs used this now wildly popular superfood in regenerative medicine as a poultice for gunshot wounds to promote healing and reduce inflammation.¹ These restorative qualities of the *Salvia hispanica* seeds are a result of mucilaginous polysaccharide. Topical application provides intense, long-term moisturization and a persistent and perceivable sensation of skin smoothing and soothing properties.

Code Number: 16882

INCI Name:

Salvia Hispanica Seed Extract

INCI Status: Conforms

REACH Status: Complies

CAS Number: 93384-40-8

EINECS Number: 297-250-8

Origin: Botanical

Processing:

GMO Free

No Ethoxylation

No Irradiation

No Sulphonation

Additives:

Natural Antimicrobial: Lactobacillus Ferment

Preservatives: None

Antioxidants: None

Other additives: None

Solvents Used: Water

Appearance: Hazy Viscous, Light Yellow to Amber Gel

Soluble/ Miscible: Water Soluble
90.9% Biodegradability

Microbial Count: <100 CFU/g,
No Pathogens

Suggested Use Levels: 1.0 -10.0%

Suggested Applications:

Wound Healing, Anti-Inflammatory, Film-Forming, Skin Care, Hair Care, Natural Cosmetics, Antioxidant

Benefits of **Phytofuse Rejuvenate®**:

- Anti-Inflammatory
- Wound Healing
- Natural Film-Former
- Intense Antioxidant
- Pro-Collagen Synthesis

Phytofuse Rejuvenate®

Salvia hispanica is near unparalleled in regards to sustainability. *Salvia hispanica* seed mucilage allows the seed to retain enough water to germinate and grow well in semi-arid regions. Able to grow in a range of climates that span several continents, *Salvia hispanica* is very easily harvested both mechanically and by hand.

SCIENCE

Polysaccharides are long chain carbohydrates capable of stabilizing biomolecules and assemblages, such as cell wall membranes. Polysaccharides, such as hyaluronic acid, are sought after for their unparalleled film forming and hydration capacities in personal care formulas. Additionally, they contribute to the skin's natural ability for repair and renewal. Decreased elasticity and the development of fine lines and wrinkles is a result of damage to the skin's protective barrier, a natural loss of water, and a decline in polysaccharide concentration on the skin. The barrier function of the skin is affected by insult and injury from several factors including environmental stress and transepidermal water loss. Exposure to ultraviolet radiation and environmental pollution can disrupt the skin's natural moisture levels. Scaly, dry skin is a direct result of an abnormal skin barrier caused by low moisture levels. Damaged skin needs to be protected at the surface and allowed to heal from within. The film forming capacities of polysaccharides defend the skin barrier and promote repair.

Current data suggests high molecular weight polysaccharides, such as β -Glucans, have potent immunomodulatory effects that regulate and improve the efficiency of the immune system.^{3,4} Inflammation in the skin is attributable to Interleukin-6 (IL-6), a proinflammatory cytokine known to play a role in immunology and aging. 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. Any immunomodulatory effects are a result of the unique polysaccharide structure. Based on the lab work and data reported, Active Concepts believes the polysaccharides in **Phytofuse Rejuvenate®** decrease the production of IL-6 and reduce inflammation, which can decrease signs of aging and reduce the appearance of fine lines and wrinkles.

Chia seed mucilage is a complex carbohydrate that is secreted when the seed comes into contact with water, generating a viscous solution. Extracted mucilage exhibits thixotropy, which is a time-dependent, shear thinning property. This is the reason the mucilage is highly viscous under static conditions, but becomes less viscous when agitated or stressed. This thixotropic nature contributes to the enhanced tactility of **Phytofuse Rejuvenate®** in formulations which gives the material a wide range of applications in skin and hair care products. Through a proprietary process, Active Concepts isolated this mucilage and bio-transformed the polysaccharides via fermentation yielding a much higher molecular weight species. This resulting natural polysaccharide is responsible for the notable wound healing, anti-inflammatory, and film forming properties of **Phytofuse Rejuvenate®**.

BENEFITS

Phytofuse Rejuvenate® is a natural alternative for the current carbohydrate chemistry seen in the medical industry for the creation of synthetic scaffolding that promotes wound healing. The isolated polysaccharides extracted from the *Salvia hispanica* seed have demonstrated their ability to aid in cell proliferation and thus impart wound healing and anti-inflammatory properties to the skin. A functional, active ingredient that improves the slip and cushion in finished formulations, **Phytofuse Rejuvenate®** soothes the skin and provides antioxidant and moisturization benefits. **Phytofuse Rejuvenate®** is ideal for skin and hair care formulations, helping to increase moisture levels and provide protection from environmental stressors.

Phytofuse Rejuvenate®

EFFICACY

Wounded tissue has a cascading effect, starting with 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.

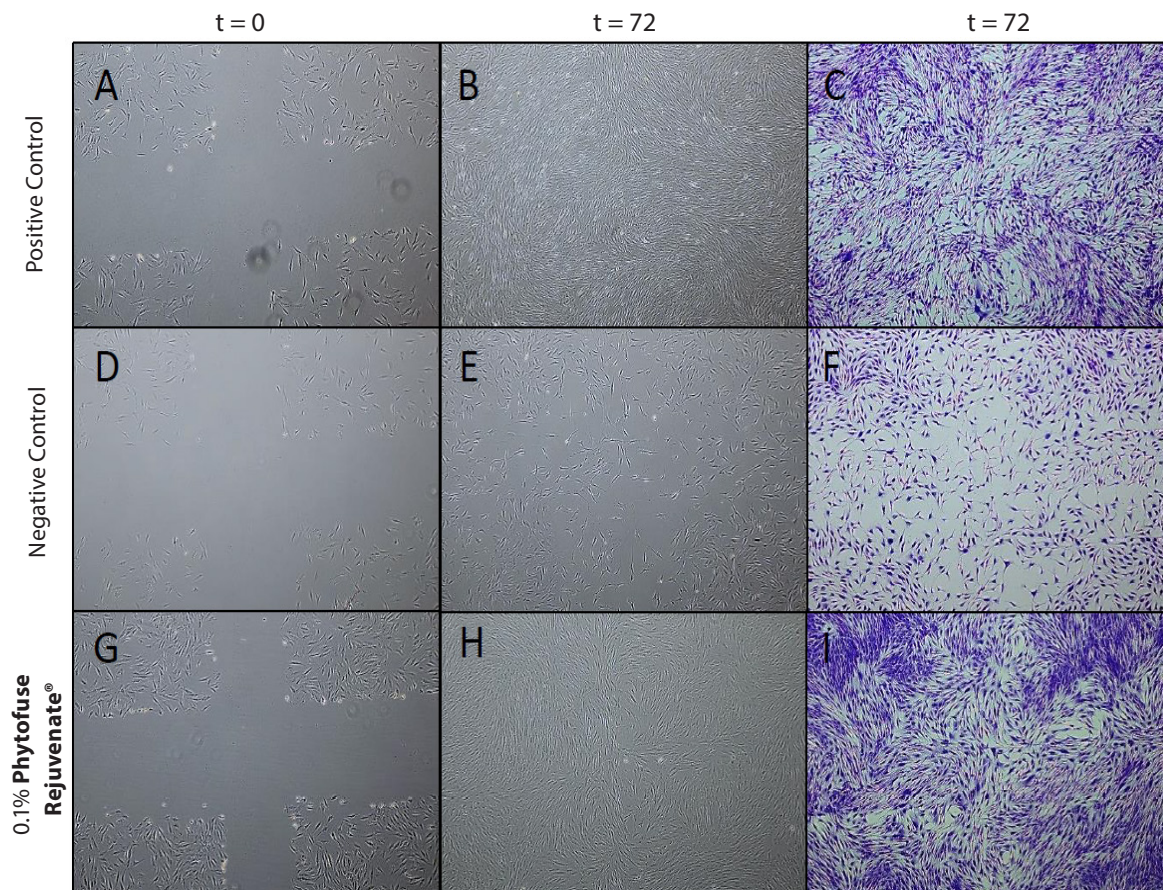


Figure 1. Images at t=0 hours (A, D, G) and t=72 hours (B, E, H) for **Phytofuse Rejuvenate®**, positive control (EGF-1), and negative control (SFM). At experiment completion (t=72 hours), cells were fixed in paraformaldehyde and stained with crystal violet (C, F, I).

From the results illustrated in Figure 1, **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. Results from this assay suggest that the product has wound healing abilities and cell proliferation properties.

Phytofuse Rejuvenate® capitalizes on the isolated polysaccharides extracted from the *Salvia hispanica* seed mucilage as a natural approach to protective and regenerative skin care. **Phytofuse Rejuvenate®** allows formulators to achieve multiple potent benefits for skin and hair utilizing a plant-derived material, revitalizing and repairing damaged skin while providing softening and emollient benefits for a luxuriant appearance. **Phytofuse Rejuvenate®** was designed to be moisturizing and soothing, as well as capable of providing enhanced aesthetics.

Phytofuse Rejuvenate®

Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in **Phytofuse Rejuvenate®**-treated *in-vitro* cultured human dermal fibroblasts. As shown in Figure 2, **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. This data suggests that **Phytofuse Rejuvenate®** enhances soothing and anti-aging properties at normal use concentrations.

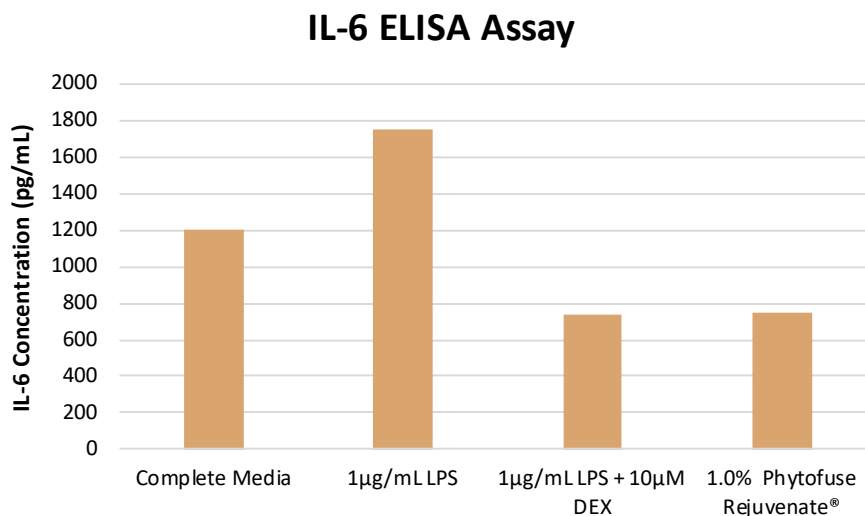


Figure 2. **Phytofuse Rejuvenate®**-treated fibroblasts IL-6 concentrations and percent change

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **Phytofuse Rejuvenate®**. As shown in Figure 3, **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 and that **Phytofuse Rejuvenate®** is capable of providing antioxidant properties.

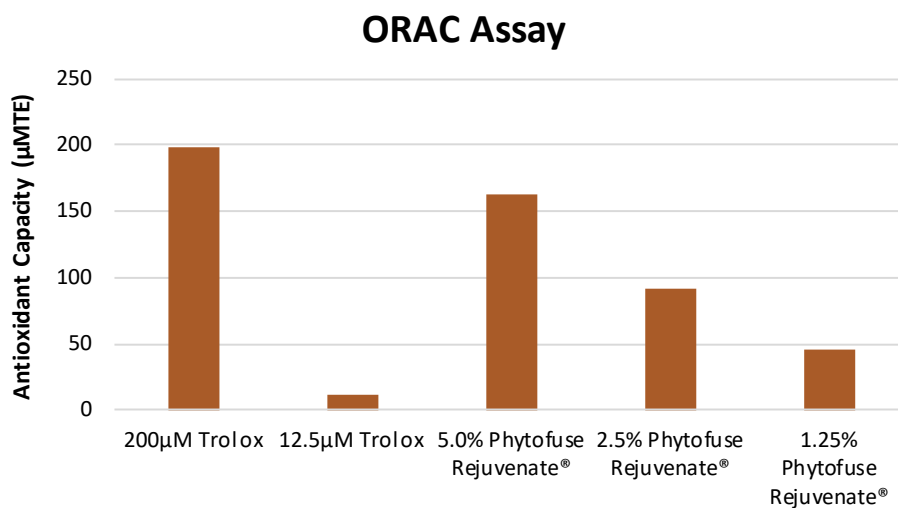


Figure 3. Antioxidant capacities of **Phytofuse Rejuvenate®**

Phytofuse Rejuvenate®

An *in-vivo* moisturization assay was conducted over a period of four weeks to evaluate the moisturizing ability of **Phytofuse Rejuvenate®**. Initial readings were taken, followed by measurements after 24 hours, one week, two weeks, three weeks, and four weeks respectively, using an Impedance Meter. This piece of equipment employs an impedance-based electronic sensing system to evaluate conductance. Results in Figure 4 demonstrate that when comparing the emulsion containing 2.0% **Phytofuse Rejuvenate®** to the untreated skin site, moisture levels increased by 24.18% after 24 hours and remained elevated by 95.31% at the end of the four week testing period. Additionally, after four weeks of application **Phytofuse Rejuvenate®** moisturized the skin 37.41% more effectively than the base lotion alone.

Comparative Moisturization

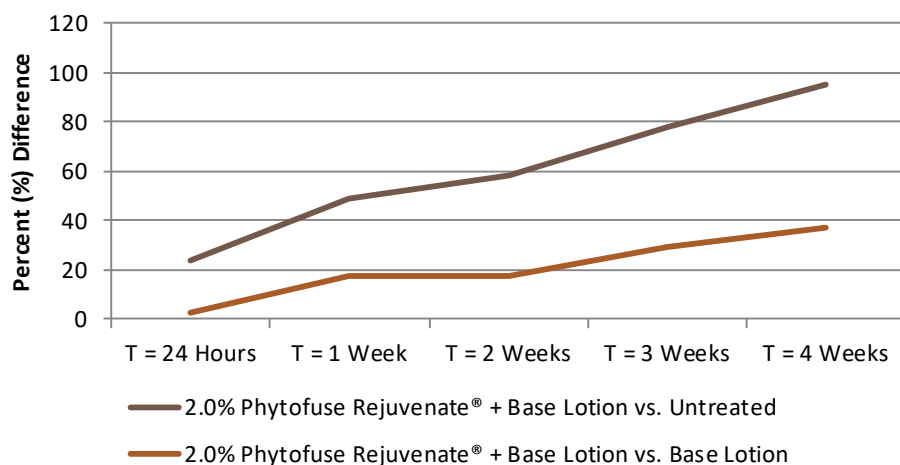


Figure 4. Improvements in moisturization

A second study was conducted to measure Transepidermal Water Loss (TEWL). TEWL is a test that accurately assesses the skin's barrier function. This characteristic is evaluated with an open chamber probe that measures the vapor diffusion gradient, or the amount of water that evaporates from the skin. Therefore, the lower the TEWL values, the better the skin's barrier function. As shown in Figure 5, 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 10.91% more effectively than the base lotion alone. After four weeks, the solution containing 2.0% **Phytofuse Rejuvenate®** demonstrated even more effective barrier protection, decreasing TEWL 35.64% better than the base lotion alone.

TransEpidermal Water Loss

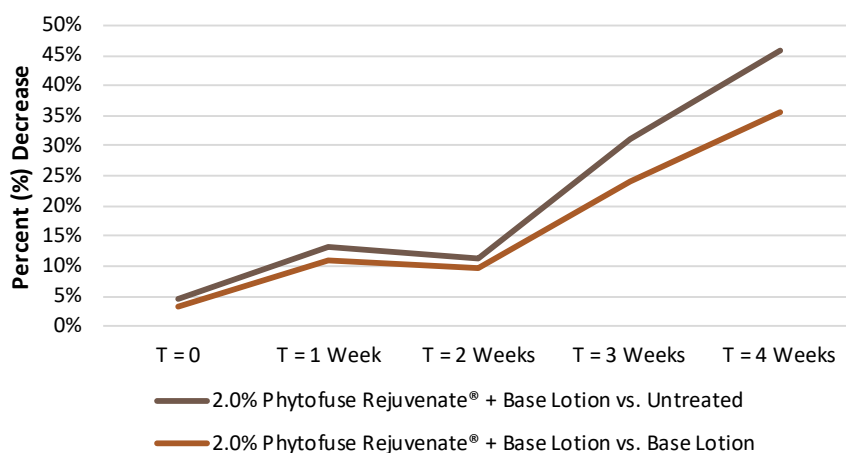


Figure 5. Comparison of transepidermal water loss

Phytofuse Rejuvenate®

The cellular viability assay was conducted to assess the ability of **Phytofuse Rejuvenate®** to increase cellular metabolic activity in cultured dermal fibroblasts. The results in Figure 6 illustrate that at concentrations of 1.0%, 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.

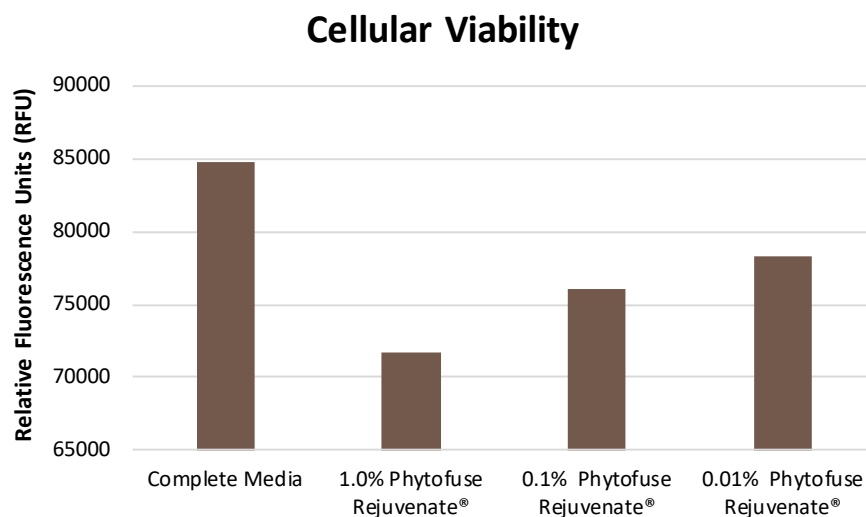


Figure 6. Cellular Metabolism of **Phytofuse Rejuvenate®**-treated fibroblasts

As shown in Figure 7, **Phytofuse Rejuvenate®** exhibits a positive effect on skin's density. In a four week efficacy study of **Phytofuse Rejuvenate®** on skin, skin density was improved by 11.82% after 24 hours and by 36.14% after 4 weeks when compared to the untreated control. When compared to the base cream **Phytofuse Rejuvenate®** improved skin density by 4.58% after 24 hours and after 4 weeks **Phytofuse Rejuvenate®** improved density by 21.01%. 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.

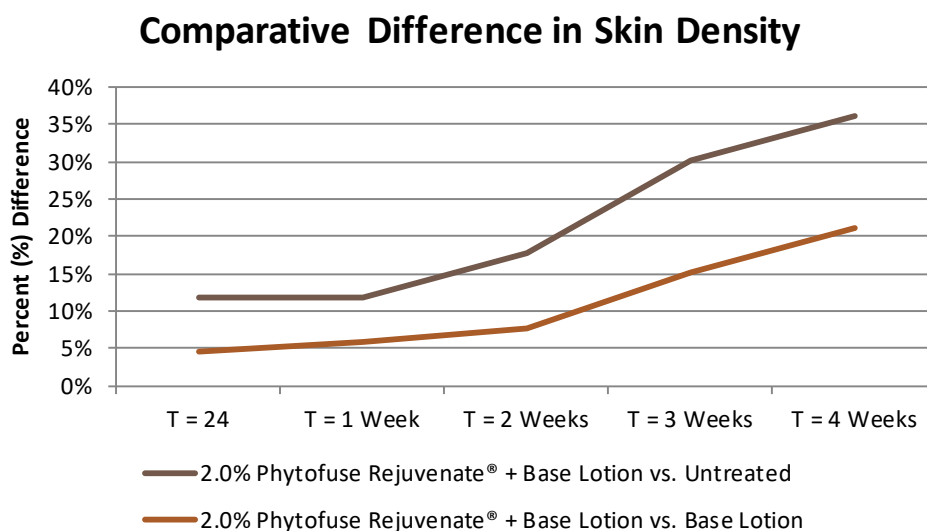


Figure 7. Collagen ultrasound results of **Phytofuse Rejuvenate®**

Phytofuse Rejuvenate®

Sirius Red/Fast Green Collagen Assay was conducted to assess the changes in collagen synthesis by **Phytofuse Rejuvenate®**-treated *in-vitro* cultured human dermal fibroblasts. As shown in Figure 8, **Phytofuse Rejuvenate®** exhibited positive collagen synthesis activity. The greatest percentage of collagen to non-collagen proteins was observed at 1.0% **Phytofuse Rejuvenate®**, as illustrated in Figure 9. The results of this assay show an increase in the percent of collagen to non-collagen proteins that correlates to an increase in the concentration of **Phytofuse Rejuvenate®**. The increase in collagen production may lead to improvement in the dermal-epidermal junction integrity as well as an improved scaffolding matrix. For these reasons, we can assume **Phytofuse Rejuvenate®** is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.

Sirius Red Fast Green Assay

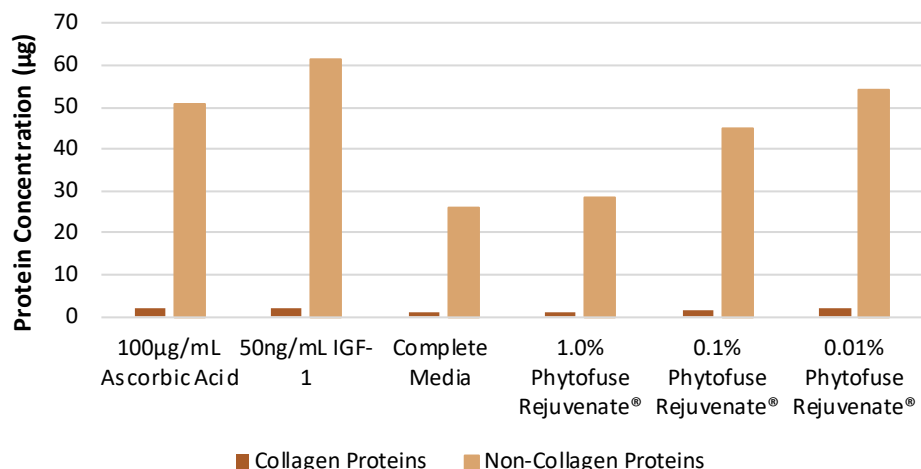


Figure 8. Collagen and non-collagen protein concentrations

Sirius Red Fast Green Assay

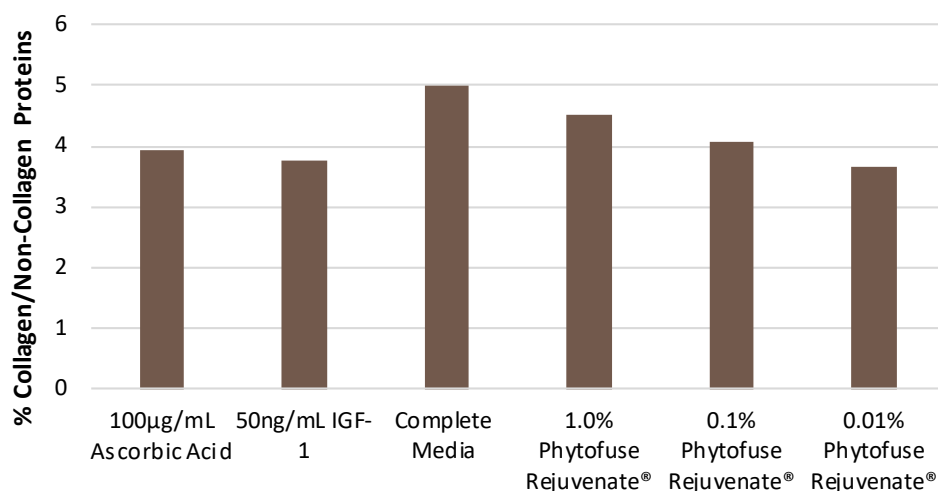


Figure 9. Percent collagen compared to non-collagen proteins

Phytofuse Rejuvenate® was tested in order to quantify a new anti-pollution standard concerning common pollutants that are >2.5µm in size. **Phytofuse Rejuvenate®** was applied to the skin and then contaminated with a premeasured amount of activated charcoal (>2.5µm size particles). It was then washed using a controlled amount of water in order to quantify **Phytofuse Rejuvenate®**'s ability to inhibit these particles from remaining on the skin. These results were compared against an untreated control and can be seen below, translated from a histogram denoting color change (lower is better, indicates skin tone).

Phytofuse Rejuvenate®

Inhibition of PM 2.5

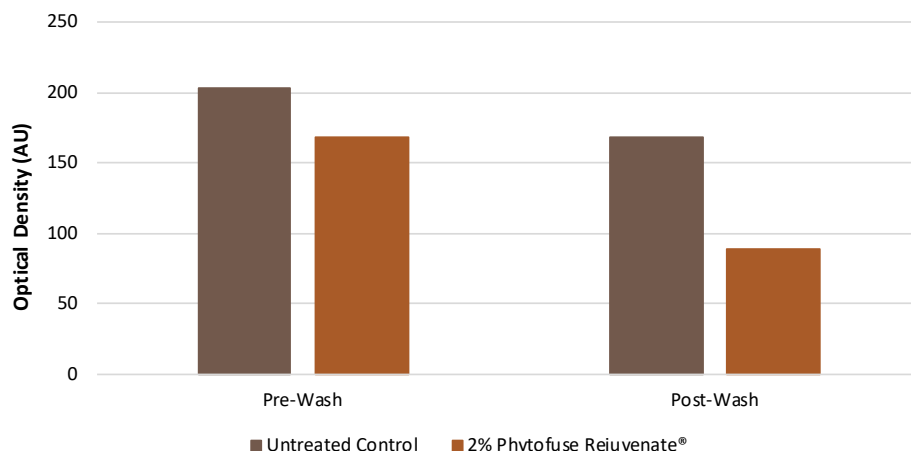


Figure 10. Ability to inhibit accumulation of particles >2.5µm in size on the skin

It can be clearly seen in Figure 12 that **Phytofuse Rejuvenate®** is able to effectively prevent the deposition of invasive PM 2.5 particles into the skin's fine lines and wrinkles. While in the untreated control group, it is depicted that the particles are able to easily penetrate the skin's fine lines, and remain there even after thorough washing.

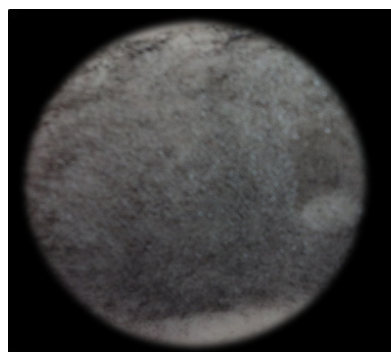


Figure 11. Phytofuse Rejuvenate® Pre-Wash

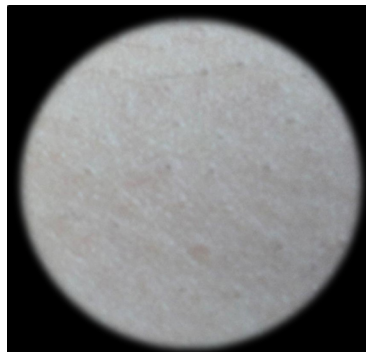


Figure 12. Phytofuse Rejuvenate® Post-Wash

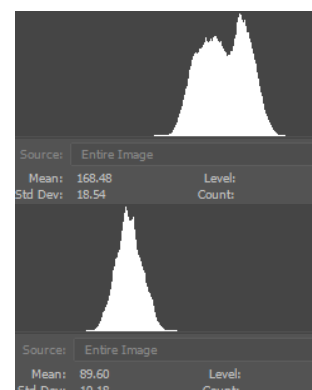


Figure 13. Treated Histograms



Figure 14. Untreated Control Pre-Wash

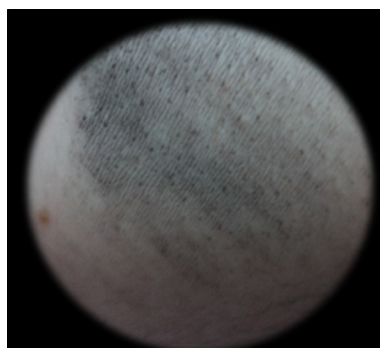


Figure 15. Untreated Control Post-Wash

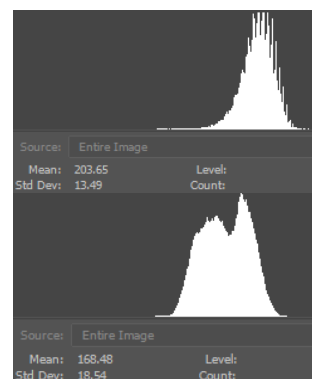


Figure 16. Untreated Histograms

References

- 1) Cahill, Joseph. 2005. Journal of Ethnobiology. Human selection and domestication of chia (*Salvia hispanica* L.). 25(2): 155-174.
- 2) Adams, James. 2014. International Journal of Genuine Traditional Medicine. What can traditional healing do for modern medicine. 4(2): 1-6.
- 3) Tzianabos, Arthur. 2000. Clinical Microbiology Review. Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. 13(4): 523-533.
- 4) Novak et al. 2008. Journal of Immunotoxicology. Beta-glucans, history, and the present: immunomodulatory aspects and mechanisms of actions 5(1): 47-57.