

Phytofuse Renew®



Resurrection Plant
spring back to life
functional active
ingredient with elegant feel
antioxidant, protectant
film-former, moisturizing, soothing, wound healing

BACKGROUND

Selaginella lepidophylla, also called the Rose of Jericho, is a botanical native to the Chihuahuan Desert in Mexico. The desert provides a unique set of challenges to plant life, such as extremely low levels of humidity and elevated temperatures year round. The Rose of Jericho is mostly known for its ability to withstand almost total desiccation for long periods of time, and then fully recover with exposure to elevated moisture levels. This is how the phrase “Resurrection Plant” became an alternate name for this plant. The plant’s secondary metabolites consist of complex enzymes and stress response elements that work synergistically to prevent significant damage during times of dessication and work to promote repair during rehydration.

The plant’s ability to spring back to “life” is a result of its highly adaptive Moisture Retention Complex. Active Concepts has sustainably isolated this Moisture Retention Complex, through advances in plant tissue culture and controlled growth conditions, to create **Phytofuse Renew®**. Topical application provides intense, long-term moisturization and a persistent and perceivable sensation of its skin smoothing and soothing properties. Incorporation of Phytofuse Renew® into skin or hair applications improves the tactile properties, while delivering the intense Moisture Retention Complex perfected by the Rose of Jericho, over thousands of years, by adapting to life in the desert.

SCIENCE

During desiccation, the Resurrection Plant is exposed to two different types of stress, mechanical and oxidative. **Mechanical stress** is the tension within the plasma membrane and cell wall that occurs due to a loss of turgor pressure. To prevent the possible rupture of the plasma membrane as a result of this stress, the plant has **employed a strategy** in which it folds the cell wall. Folding forces the plant to curl and wilt, **making it appear lifeless**. However, upon

Code Number: 16586

INCI Name: Selaginella

Lepidophylla Extract

INCI Status: Approved

REACH Status: Complies

CAS Number: 90106-73-3

EINECS Number: 290-298-0

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 Hazy Yellow
to Amber Gel

Soluble/ Miscible: Water Soluble &
Soluble in Hydroalcoholic Systems

Ecological Information:

87.50% Biodegradability

Microbial Count: <100 CFU/g,
No Pathogens

Suggested Use Levels: 1.0 – 10.0%

Suggested Applications:

Moisturizing, Antioxidant, Enhanced
Aesthetics, Protectant, Soothing,
Wound Healing

Benefits of Phytofuse Renew®:

- Enhances Aesthetics of Final Formulas
- Intense Moisturizing Benefits
- Perceivable Sensorial Attributes
- Improved Barrier Function
- Antioxidant Protection

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exposure to **high moisture levels**, the plant **bursts open** revealing the **appearance of life**. The second type of stress, **oxidative stress**, is responsible for triggering the production of the **moisture-retentive system** of the Resurrection Plant. This is comprised of polysaccharides, enzymes, stress response proteins and polyphenols. The **activity and stability** of each compound is completely dependent on another. Thus, the **compounds work synergistically to protect and repair** the plant. Polysaccharides are long chain carbohydrates capable of stabilizing biomolecules and assemblages, such as cell wall membranes. Additionally, they have the ability to retain water, keeping the plant hydrated during droughts to prevent complete desiccation. During rehydration, polysaccharides mobilize water through the system and increase cell metabolism to **accelerate the repair process**.

The enzymes involved in the process serve the role of boosting carbohydrate metabolism to **repair the damage** caused during desiccation. This indicates the activity of the polysaccharides is dependent on the activity of the enzymes. However, the integrity of the enzymes is generally compromised during desiccation. This is caused by protein aggregation, a biological phenomenon in which miss-folded proteins accumulate and clump together, reducing the protein activity. Nevertheless, one of the main roles of the stress response protein in the plant system is to **prevent protein aggregation**. Stress proteins behave as molecular chaperones that bind to the proteins to maintain them in a folded, competent state, preventing irreversible aggregation and inactivation while promoting repair after damage. Yet, this effect can only be achieved synergistically in the presence of the non-reducing disaccharides in the plant system.

BENEFITS

A functional, active ingredient that improves the slip and cushion in finished formulations, **Phytofuse Renew®** soothes the skin and provides benefits such as moisturization and protection. This product, which imparts an immediate cooling sensation when applied, simultaneously enhances cellular proliferation to aid in wound healing. In hair care, **Phytofuse Renew®** increases the sheen of the hair while hydrating and providing antioxidant protection against environmental stressors.

Phytofuse Renew® imparts protective and moisture-retentive benefits associated with the Moisture Retention Complex devised as a stress response, and specifically, associated with the secondary metabolites produced by the Resurrection Plant. The plant's secondary metabolites consist of complex enzymes and stress response elements that work synergistically to prevent significant damage during times of desiccation and act to promote repair during rehydration. Additional benefits of the Moisture Retention Complex include **Phytofuse Renew's®** ability to deliver film-forming, water-holding and soothing effects to the skin. **Phytofuse Renew®** is a natural alternative for the current carbohydrate chemistry being used for the creation of synthetic scaffolding that promotes wound healing, both in geriatric and burn care research. The isolated and intact iridoid systems extracted from the plant have demonstrated their ability to aid in cell proliferation and thus impart wound healing properties to the skin. **Phytofuse Renew®** is ideal for skin and hair care, helping increase moisture levels and provide protection from the elements.



EFFICACY DATA

A series of *in-vivo* studies were conducted to evaluate the ability of **Phytofuse Renew®** to provide active benefits to the skin. Ten volunteers (M/F) between the ages of 27 and 56, known to be free of any skin pathologies, participated in these studies. Volunteers were asked to apply an O/W emulsion containing 2.0% **Phytofuse Renew®** to their volar forearms twice a day for three weeks. Measurements were taken using the Dermalab Combo. For added perspective, measurements of an untreated test site and a site treated with the O/W emulsion with no additives were also recorded.

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The first study was conducted to measure the moisturizing ability of the product. Initial readings were taken, followed by measurements after 24 hours, one week, two weeks, and three weeks, respectively, using an Impedance Meter. This piece of equipment employs an impedance-based electronic sensing system to evaluate conductance. Results indicated that after 24 hours, the solution containing 2.0% **Phytofuse Renew®** moisturized the skin 17% more effectively than the base lotion alone, while enhancing hydration levels 26% better than the base lotion alone after three weeks of application. When comparing the emulsion containing 2.0% **Phytofuse Renew®** to the untreated skin site, moisture levels increased by 71% after 24 hours and remained elevated by 95% at the end of the three week testing period.

Comparative Moisturization

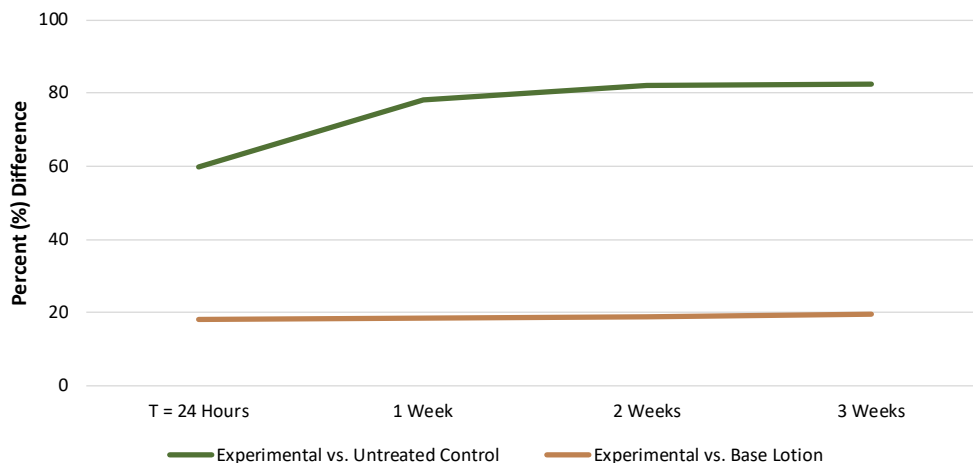


Figure 1. Improvements in moisturization following application of the test materials.

The 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 2, results indicate continuous improvements in the barrier of the skin throughout the 3 week test period. After one week, the solution containing 2.0% **Phytofuse Renew®** decreased TEWL 12% more effectively than the base lotion alone. After three weeks, the solution containing 2.0% **Phytofuse Renew®** demonstrated even more effective barrier protection, decreasing TEWL 19% better than the base lotion alone.

TEWL Comparison Over Time

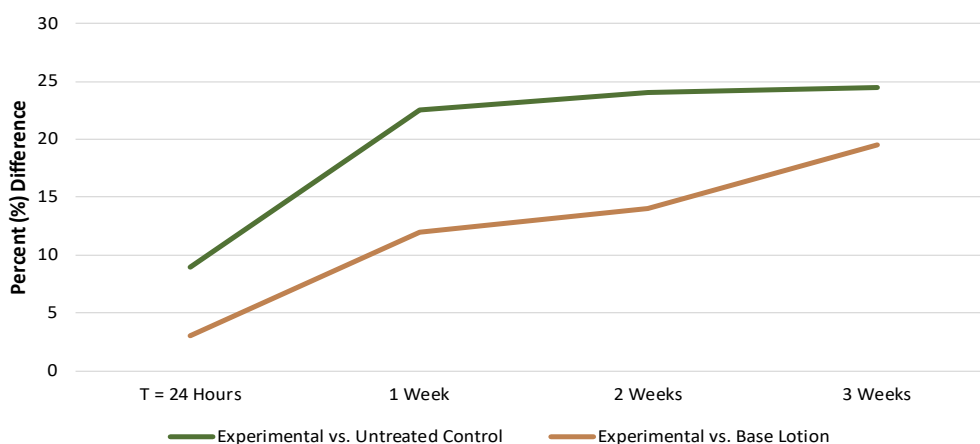


Figure 2. Comparison of percent change between test sites after application of the test materials for a 3 week.

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The third *in-vivo* study was conducted to evaluate the density of the stratum corneum which directly relates to the ability of **Phytofuse Renew®** to repair damage by optimizing the production of structural proteins and promoting their proper folding. Improvements in the structural matrix significantly reduce the appearance of fine lines and wrinkles, promoting a youthful appearance. Improvements in skin density were analyzed using an ultrasound (Rotating 20 MHz X-tal, 3.7 mm penetration) on the test areas.

Skin Density Comparison Over Time

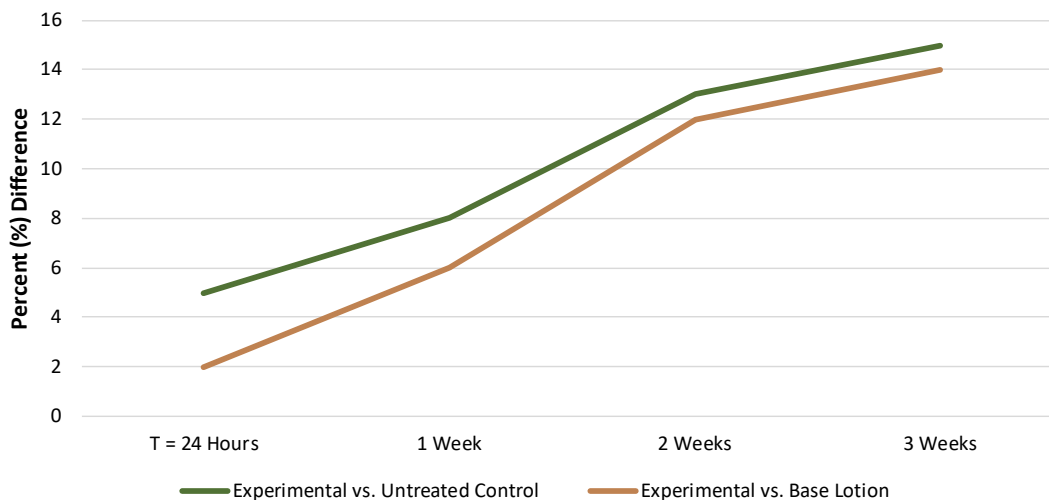


Figure 3. Improvements in Skin Density following application of the test materials after a period of 3 weeks.

Results indicate that the solution containing 2.0% **Phytofuse Renew®** improves skin density 6.0% better than using only a base lotion after one week and improves skin density 14% better after three weeks than using the base lotion alone. An ORAC Assay was conducted to evaluate **Phytofuse Renew's®** ability to reduce oxidative stress. This assay is based upon the effect of peroxy radicals generated from the thermal decomposition of 2,2'-azobis-2-methylpropanimidamide dihydrochloride on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. Results indicate that the product provides intense protection against Reactive Oxygen species comparable to Trolox, an analogue of Vitamin E.

ORAC Assay

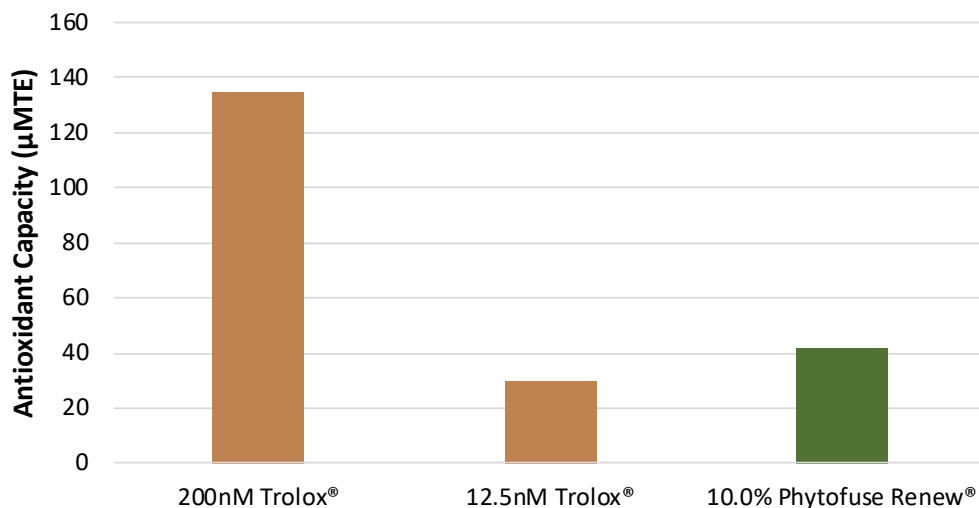


Figure 4. Comparison of the Antioxidant Capacity of **Phytofuse Renew®** and Trolox®.

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Phytofuse Renew® was tested in order to quantify a new anti-pollution standard concerning common pollutants that are $>2.5\mu\text{m}$ in size. **Phytofuse Renew®** was applied to the skin and then contaminated with a premeasured amount of activated charcoal ($>2.5\mu\text{m}$ size particles). It was then washed using a controlled amount of water in order to quantify **Phytofuse Renew®**'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).

Inhibition of PM 2.5

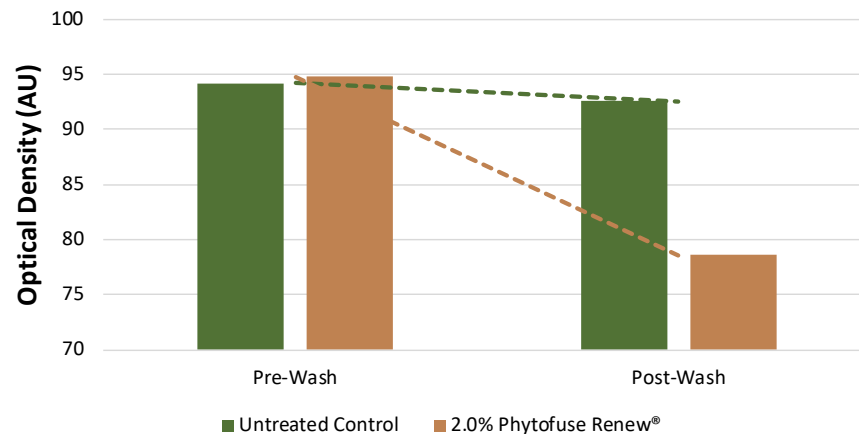


Figure 5. Ability to inhibit accumulation of particles $<2.5\mu\text{m}$ in size on the skin.

It can be clearly seen in Figure 6 that **Phytofuse Renew®** 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 shown that these particles are able to easily penetrate these lines, and remain there even after thorough washing.

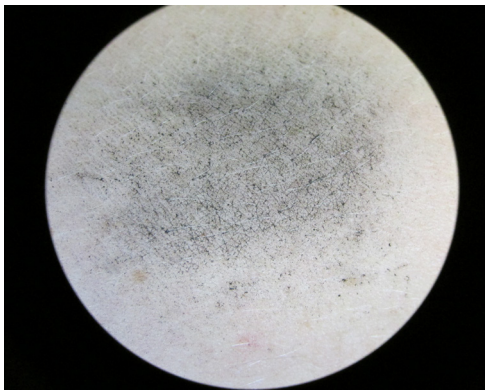


Figure 6. Phytofuse Renew® pre-wash

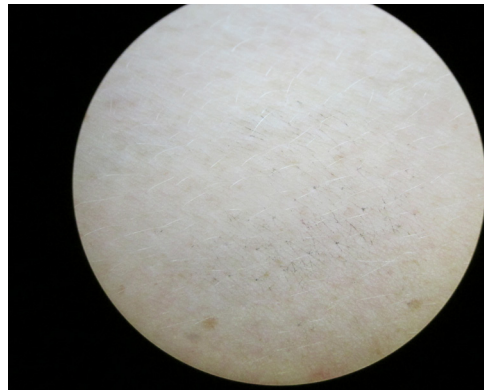


Figure 7. Phytofuse Renew® post-wash

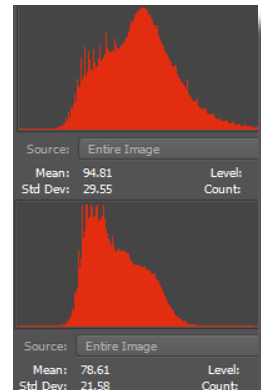


Figure 8. Phytofuse Histograms

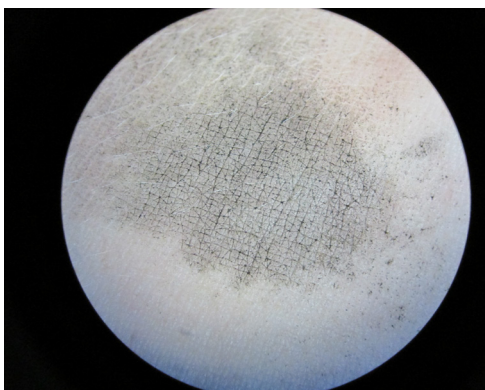


Figure 9. Untreated control pre-wash

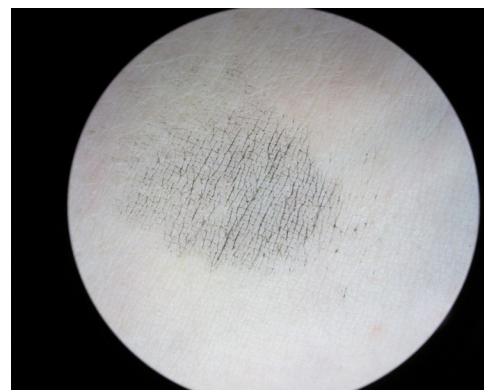


Figure 10. Untreated control post-wash

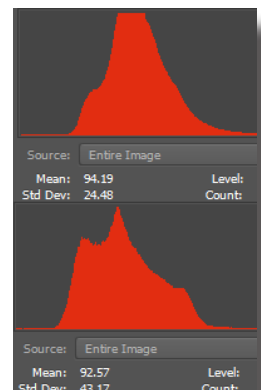


Figure 11. Untreated Histograms

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An *in-vitro* Interleukin-6 ELISA study was conducted to assess the changes in IL-6 levels in **Phytofuse Renew®** treated cultured human dermal fibroblasts. Interleukin-6 is a pro inflammatory 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. Results indicate **Phytofuse Renew®** exhibits 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.

IL-6 ELISA

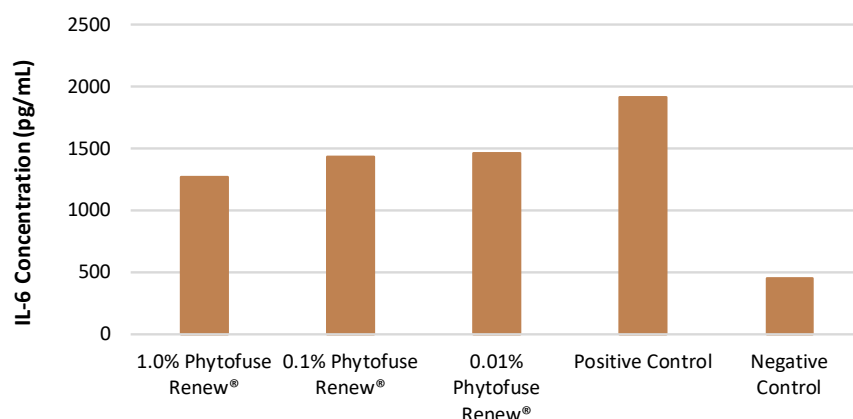


Figure 12. IL-6 concentrations.

An *en-vivo* study was conducted to evaluate the ability **Phytofuse Renew®** to protect hair from pollution. The deleterious effects of pollution in skin and hair care has become a new frontier for anti-aging active ingredients. Environmental pollutants are results of automobile exhaust gas, industrial emissions, and even emissions from simple household chores such as cooking and cleaning. Hair is subject to these environmental aggressions as well as UV irradiation and, unlike the skin, hair is quite vulnerable and lacks self-protection mechanisms. Exposure to environmental pollution can result in dry, brittle hair with decreased strength and elasticity.

Hair swatches were treated and exposed to cigarette smoke, and peroxidation of hair lipids were assessed using a Malonaldehyde (MDA) Assay. The Malondialdehyde (MDA) assay is useful for quantitatively measuring the end product of lipid peroxidation and determining oxidative stress. MDA is frequently used as a bio marker for oxidative stress and, in this case, lipid peroxidation (the breaking down of lipids) due to environmental stress. An increase in MDA indicates an increase in lipid peroxidation and oxidative stress.

Malonaldehyde Lipid Peroxidation Average MDA Values

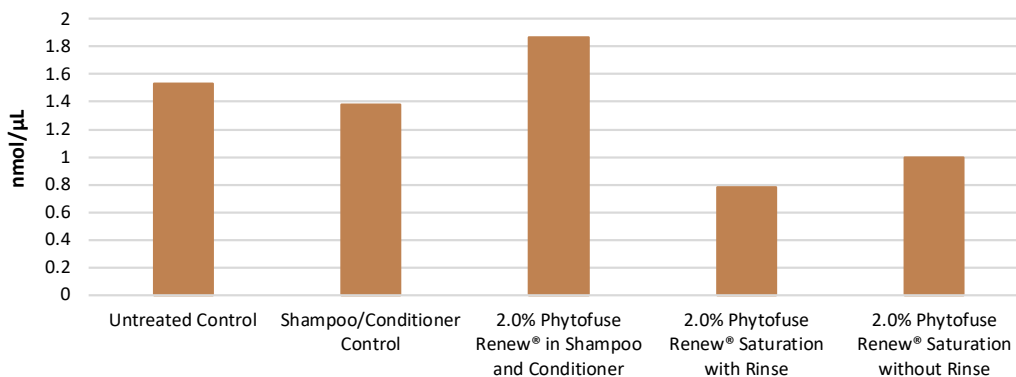


Figure 13. Average values of MDA.

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In this study, **Phytofuse Renew®** was tested to evaluate its effects on the inhibition of lipid peroxidation of hair samples exposed to air pollution. At a concentration of 2.0%, **Phytofuse Renew®** demonstrated significantly lower levels of MDA than the untreated controls. It can therefore be concluded that at normal use concentrations **Phytofuse Renew®** can be used as a hair pollution protection active ingredient.

A scratch assay was conducted to assess the wound healing properties of **Phytofuse Renew®** treated, *in-vitro* cultured human dermal fibroblasts. **Phytofuse Renew®** 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. **Phytofuse Renew®** was designed to be moisturizing and soothing, and provide enhanced aesthetics. With the present study we can also be confident that this product has healing abilities and cell proliferation properties.

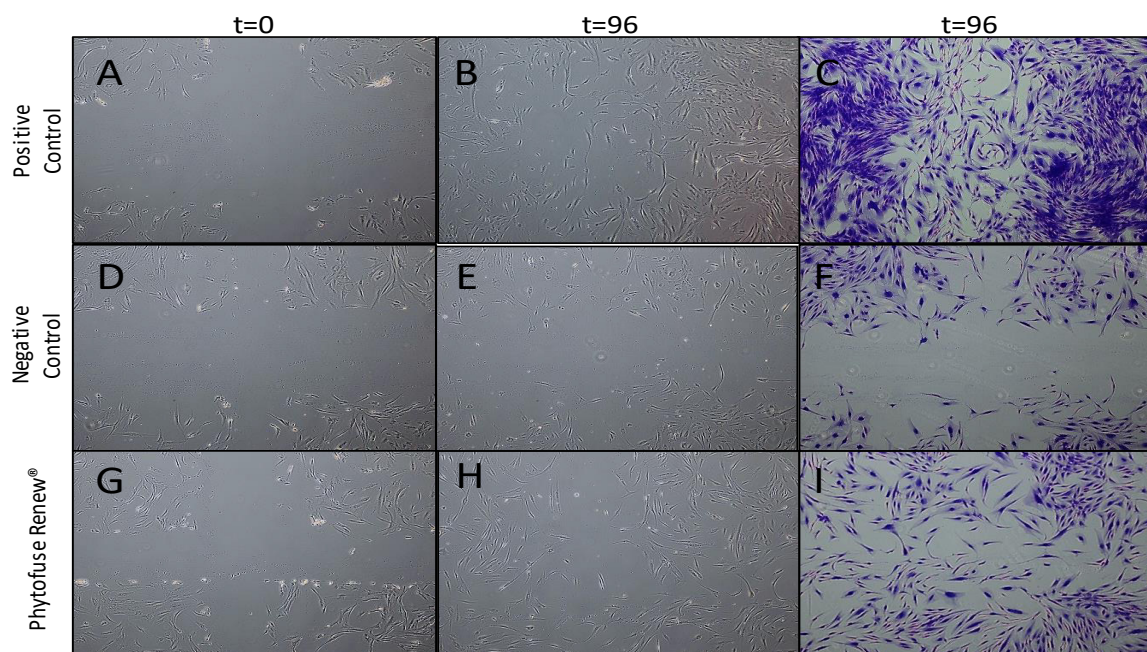


Figure 14. Images at t=0 hours (A, D, G) and t=96 hours (B, E, H) for **Phytofuse Renew®**, positive control, and negative control. At experiment completion (t=96 hours), cells were fixed in paraformaldehyde and stained with crystal violet (C, F, I).

Capitalizing on the **adaptive strategies of exotic plant** is at the forefront of today's approach to protective and regenerative skin care. **Phytofuse Renew®** allows formulator's to achieve multiple potent benefits for skin and hair utilizing a **plant-derived** material, rejuvenating and repairing damaged skin while providing softening and emollient benefits for a **luxuriant appearance**.