

# Phyto-Biotics Quercus



## BACKGROUND

The cosmetic market has seen a considerable uptick in the interest of stem cells in the last decade. Trending now, the cosmetic and personal care industries provide stem cell technologies which 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 Quercus™ capitalizes on specific activity of the phenolic metabolites produced by Quercus Alba, or White Oaks, to provide the cosmetic market with a plant-derived, stem cell ingredient designed to impart antioxidant, soothing, antimicrobial and anti-aging benefits while enhancing cellular metabolism.

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: 16588**

**INCI Name:** Quercus Alba Bark Extract  
**INCI Status:** Approved  
**REACH Status:** Complies  
**CAS Number:** 68917-11-3  
**EINCS Number:** 272-838-7

**Origin:** Botanical

**Processing:**

- GMO Free
- No Ethoxylation
- No Irradiation
- No Sulphonation

**Additives:**

- Preservatives: None
- Antioxidants: None
- Other additives: None

**Solvents Used:** Water

**Appearance:** Hazy Liquid

**Soluble/ Miscible:** Water Soluble

**Ecological Information:** Sustainable

**Microbial Count:** < 100 opg,  
No Pathogens

**Suggested Use Levels:** 1.0 – 10.0%

**Suggested Applications:** Anti-Aging, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism

## Benefits of Phyto-Biotics Quercus:

- Stem Cell Technology
- Improves Skin Density
- Anti-Aging
- Potent Antioxidant
- Enhances Cellular Metabolism
- Improves ATP Synthesis

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## 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. Meristematic 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.

Not readily thought of as a cosmetic ingredient, the secondary metabolites of White Oak's are ideal for developing activity-specific plant stem cell materials. There are many species of oak found all over the world, however, *Quercus alba*, or White Oaks, are found primarily in North America. Due to this tree's ability to grow in a variety of environments, White Oaks are susceptible to pathogenic stress like fungi and insects. This is no match for the White Oak, which continues to flourish as a result of the secondary metabolites that have allowed this oak to evolve and ward off potential threats. The phenolic compounds, or secondary metabolites, produced by *Quercus alba* act as antimicrobials and antioxidants, in addition to promoting anti-aging by acting as a scavenger of Reactive Oxygen Species and providing soothing benefits.

Historically, the Romans used White Oak Bark as a medicinal treatment for chronic stomach issues and hemorrhages. Listed in the United States Pharmacopoeia from 1820 to 1919 and the National Formulary from 1916 to 1936, White Oak Bark was traditionally used by Native Americans and European settlers for its astringent and anti-inflammatory properties. White Oak Bark has high constituents of the phenolic compounds, tannins and quercin. With potent astringent properties, White Oak Bark Extract is known to help absorb toxins and soothe irritated, swollen skin. With natural antimicrobial properties, White Oak Bark Extracts are often used in topical applications to treat skin issues, specifically bacterial and viral infections.

The secondary metabolites, or phenolic metabolites, of interest from *Quercus alba* are tannins. Ellagitannins

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are a type of tannin known to have potent antioxidant, antiviral, antimicrobial, and anti-parasitical properties. Additionally, tannins have been shown to remove damaging oxidants and free radicals, making them ideal for anti-aging and protectant cosmetic applications. Additionally, ellagitannins may increase the cellular regeneration, resulting in faster recovery time for damaged cells. Furthermore, tannins are useful for imparting soothing benefits because of their anti-inflammatory properties.

## BENEFITS

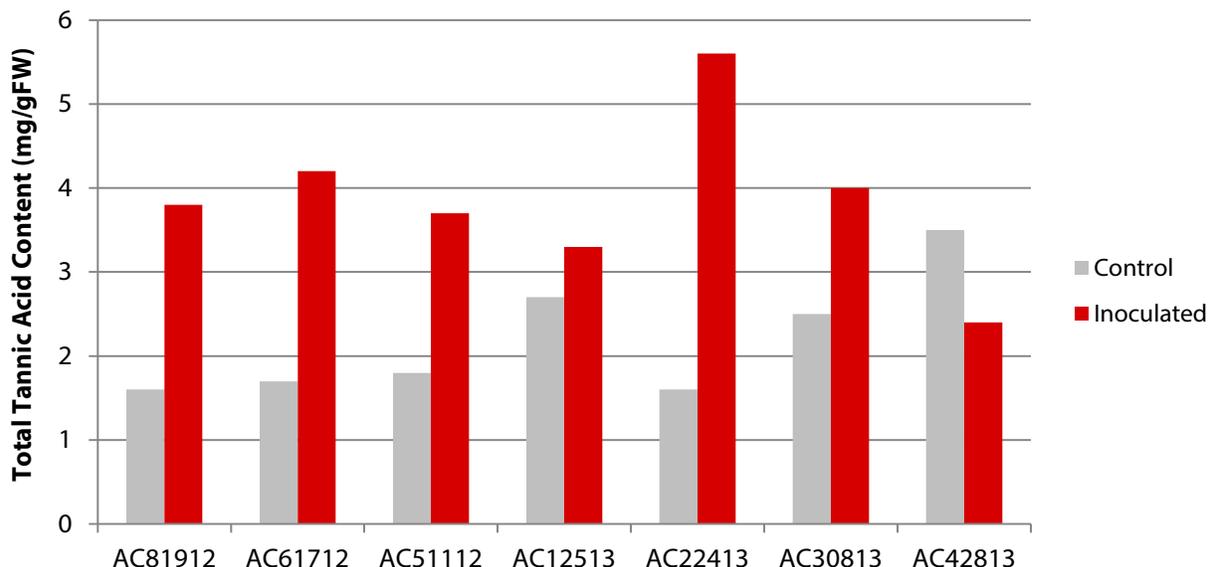
As previously mentioned, using solvent extraction from a naturally grown, a whole plant is environmentally damaging. In order to sustainably source the necessary phenolic metabolites, sourced from tannic acid, Active Concepts grows *Quercus alba*, or White Oaks, in cell culture. Using biotic stress, specifically pathogenic stress via *Leuconostoc sp.*, our formulators created Phyto-Biotics Quercus by inducing the production of tannic acid to create a plant stem cell product ideal for anti-aging cosmetic applications.

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 the market with the differentiation of plant stem cells for brand differentiation and multiple cosmetic benefits; Phyto-Biotics Quercus.

## EFFICACY

To demonstrate the improvements in tannic acid content in White Oak Bark co-cultured with *Leuconostoc sp.*, an efficacy assay was performed comparing it to un-inoculated controls. Genetically uniform, shoot-based clonal lines of *Quercus alba* 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 *Quercus alba*. After thirty (30) days, the tannic 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 tannin content in the White Oak Bark co-cultured with *Leuconostoc sp.* in comparison to the control are clearly indicated in the following graph<sup>2</sup>.

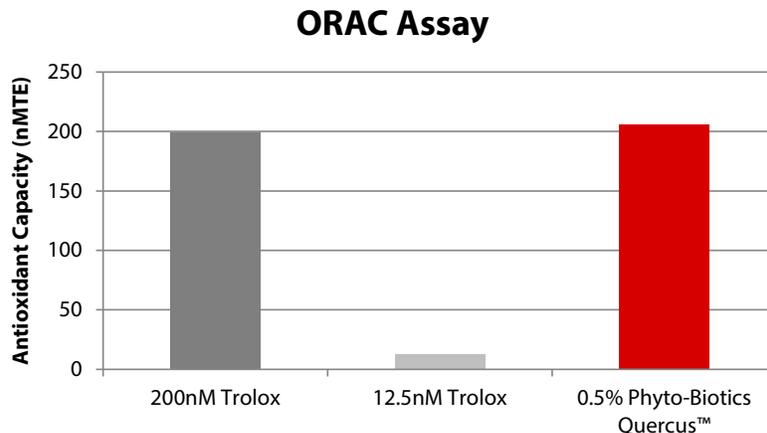
**Improvements in Tannic Acid Content in White Oak Bark Co-Cultured with *Leuconostoc sp.***



**Figure 1.** Compared to the control Tannic Acid Content increases when White Oak Bark is Co-Cultured with *Leuconostoc sp.*

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An Oxygen Radical Absorbance Capacity Assay was conducted in-house to measure the antioxidant activity of Tannic Acid. Phyto-Biotics Quercus, standardized for Tannic Acid, was compared with a Trolox Control (Vitamin E derivative and antioxidant standard in the cosmetic market).

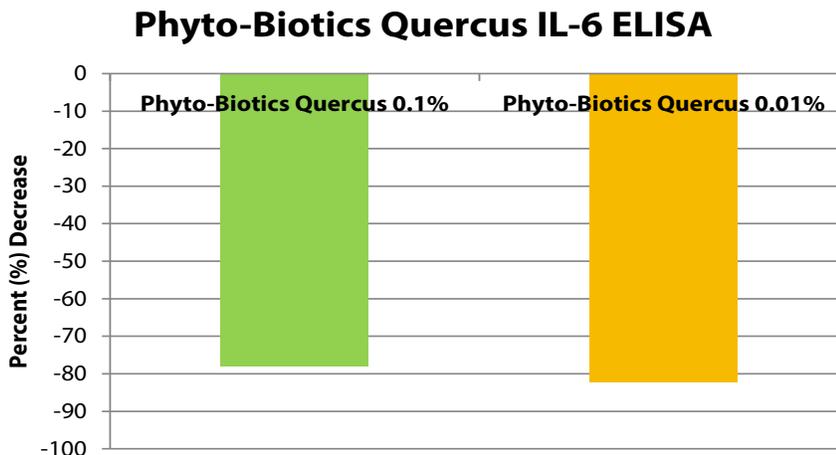


**Figure 2.** The results indicate that the Tannic Acid constituents of Phyto-Biotics Quercus provide comparable antioxidant activity to 200nM Trolox

Phyto-Biotics Quercus was designed to provide skin firming and antioxidant benefits when used in cosmetic applications. The ORAC Assay confirms that this ingredient is capable of providing functional benefits and potent antioxidant activity in skin care formulas.

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 Quercus. 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 Quercus, standardized for Tannic 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 3.** Phyto-Biotics Quercus is capable of reducing inflammatory environments

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As demonstrated from this ELISA Assay, Phyto-Biotics Quercus exhibits anti-inflammatory effects. The 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. Phyto-Biotics Quercus is assumed to be a suitable addition to cosmetic applications designed to provide soothing and anti-aging properties.

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 Quercus 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 Quercus in concentrations of 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 Quercus™ at all concentrations is able to increase cellular metabolism compared to the control.

**Cellular Metabolism of Phyto-Biotics Quercus**

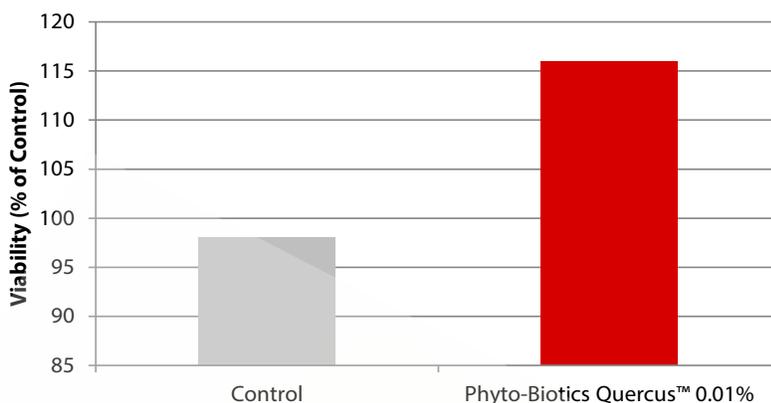
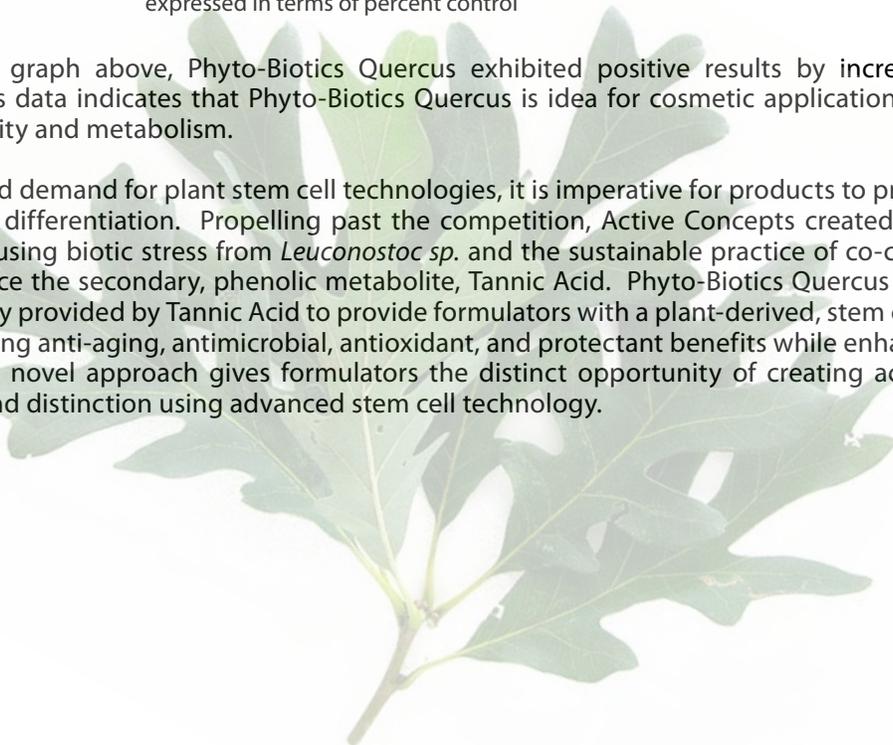


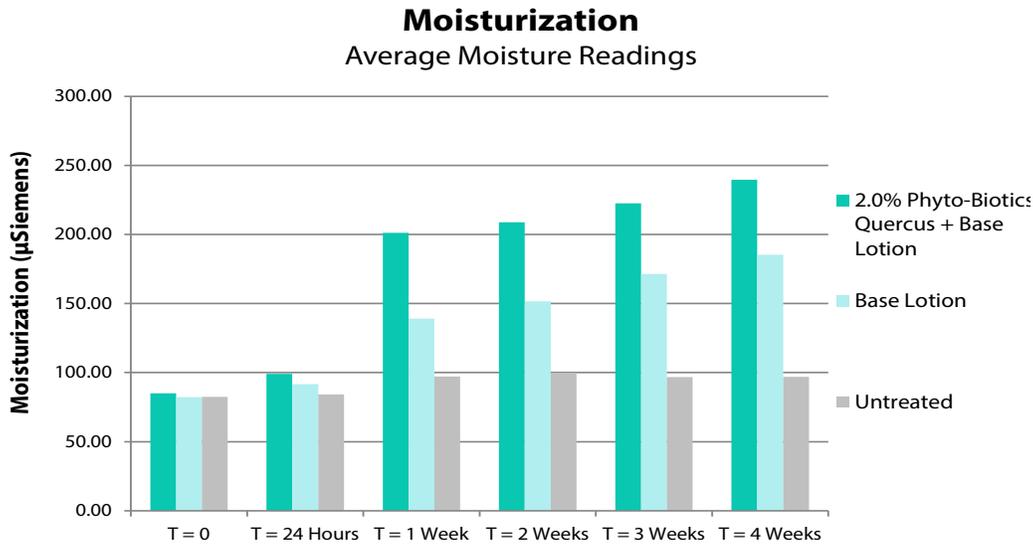
Figure 4. Cellular Metabolism of Phyto-Biotics Quercus treated in fibroblasts expressed in terms of percent control

As shown in the graph above, Phyto-Biotics Quercus exhibited positive results by increasing cellular metabolism. This data indicates that Phyto-Biotics Quercus is ideal for cosmetic applications designed to increase cell viability and metabolism.

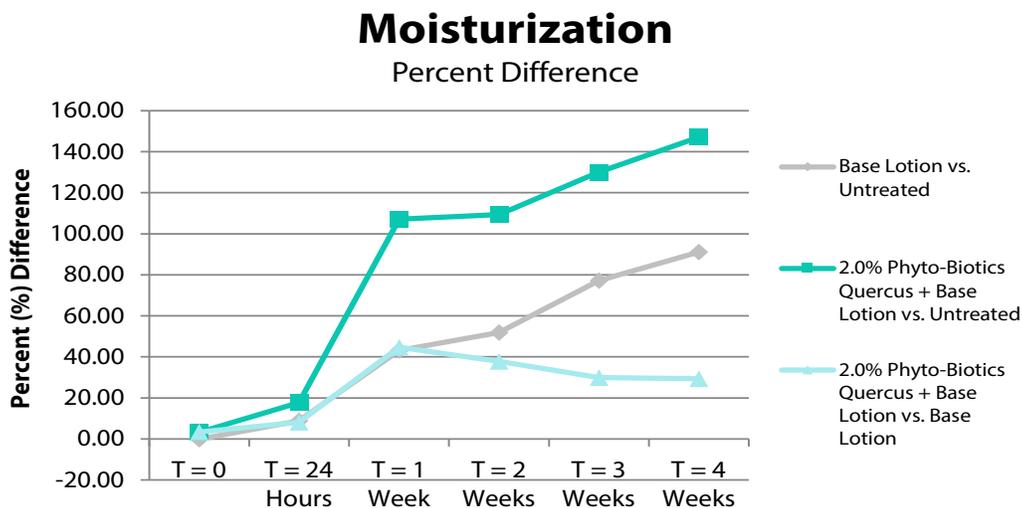
With the continued demand for plant stem cell technologies, it is imperative for products to provide specific activity for brand differentiation. Propelling past the competition, Active Concepts created Phyto-Biotics Quercus, created using biotic stress from *Leuconostoc sp.* and the sustainable practice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



**Figure 5.** Average increase in skin moisturization per test site



**Figure 6.** Percent difference in moisturization between two test sites over four weeks

As evidenced in a 4 week efficacy study of Phyto-Biotics Quercus on skin, moisture levels were improved by 17.63% after 24 hours and by 146.98% after 4 weeks when compared to the untreated control. Comparisons of the base lotion to the Experimental Lotion containing 2.0% Phyto-Biotics Quercus demonstrate the experimental material moisturized the skin 8.10% better after 24 hours. After two weeks the base lotion containing 2.0% Phyto-Biotics Quercus moisturized skin 44.69% better than the base lotion alone and was 29.30% more moisturizing than the base lotion after 4 weeks. Results indicate that Phyto-Biotics Quercus 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 Quercus 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 Quercus + Base Lotion was approximately 53.0% more moisturized than the site which received no treatment. After two weeks, the experimental test site was still yielding moisturization results that were 15.87% 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 Quercus + Base Lotion moisturized the skin 16.08% better after 24 hours and was still 13.77% more effective in moisturizing the skin when readings were taken two weeks after the applications of both test materials ceased.

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## Moisture Regression Experimental Treatment vs. Untreated

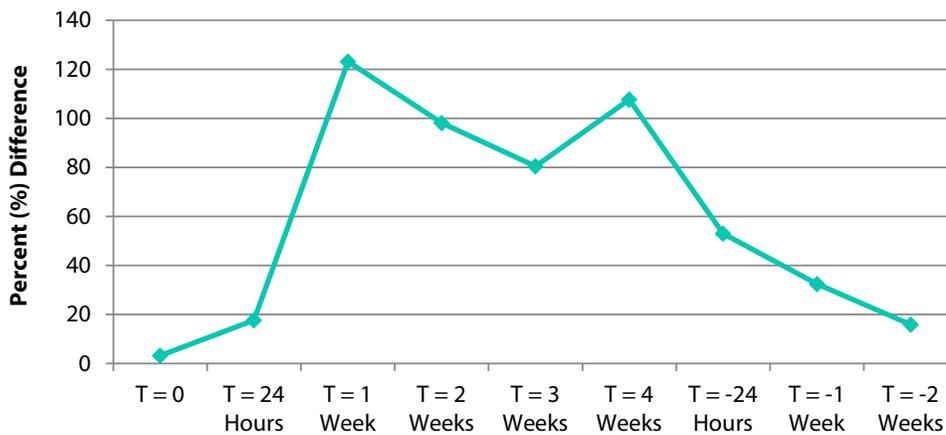


Figure 7. Regression in skin moisturization after application of experimental material ceased

As evidenced in a 4 week efficacy study of Phyto-Biotics Quercus, skin density showed a steady improvement over 4 weeks when compared to the untreated control and the base lotion. When compared to the base cream, Phyto-Biotics Quercus was more effective in improving skin density at the end of each test week. Results indicate that Phyto-Biotics Quercus is capable of consistently increasing collagen levels and skin density over time, in comparison to the base lotion which demonstrated lower or steady collagen levels each week in comparison to the initial reading.

Phyto-Biotics Quercus continues to have steady, and positive, effect on skin's density and collagen levels when used at recommended use levels.

## Average Results Per Test Site

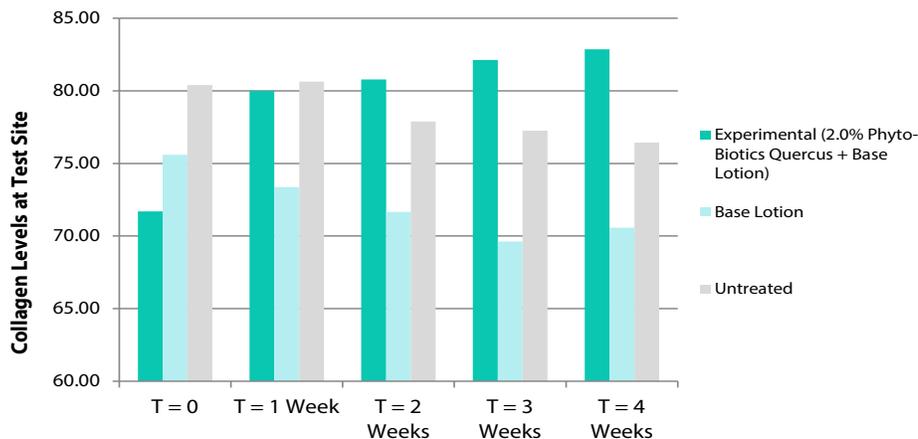


Figure 8. Skin density measured via collagen level at each test site

**References:**

- 1) Pugliese, Michael Q., BS, L.E. Stem Cells: What and Why. Derascope: The Publication of Aesthetics and Spa Therapy. Michael Q. Pugliese, BS, L.E. (2012).
- 2) Winks, M. & Schimmer, O. (1999) Modes of action of defensive secondary metabolites. Function of Plant SMs and their exploitation in biotechnology. Annual Plant Reviews. pp. 17-133. Sheffield Academic Press, Sheffield

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