Truth + Fiction + Path to Sustainability

Meristemic Cells + Brand Differentiation + Phyto-Biotics

Tomorrow’s Vision... Today!®
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On average, American women use 12 personal care products a day, and men average six products daily. That means an adult is likely to be exposed daily to 126 unique chemical ingredients in personal care products alone.¹

Environmental, social and economic impact of the personal care industry

Research shows the most significant footprints in the cosmetic industry lie with packaging and ingredients

The cumulative effects of wasteful and irresponsible habits associated with the personal care industry can significantly contribute to global warming

OUR NEGATIVE IMPACT

“Our global footprint now exceeds the world's capacity to regenerate by about 30 percent.”²

FACTS:

Extinction rates are rising by a factor of up to 1,000 above natural rates. Every hour, three species disappear. Every day, up to 150 species are lost. Every year, between 18,000 and 55,000 species become extinct. The cause: human activities. ³ ~ Ahmed Djoghlaf

Every day, an estimated 100 plant and animal species are lost to deforestation. ⁴

Many river systems approach the fate of those in China, where chiefly because of pollution 80 percent of the 50,000 kilometers of major channels can no longer support fish of any kind. ⁵
OUR RESPONSIBILITY

“The social, economic and environmental pillars of sustainability all have a place at the heart of the life cycle of cosmetic and personal care products. The integration of these elements is not a marketing tool, it represents a genuine commitment to sustainability ... at many levels of the business model.”

~ Bertil Heerink - Director-General of Cosmetics Europe

What this means?

- Personal Care and Cosmetic companies need to think and act sustainably
- Includes sourcing, producing, manufacturing, packaging, and distributing
- Attention to health and well-being of consumers

WHAT IS SUSTAINABILITY?

Development that meets the needs of the present without compromising the ability of future generations to meet their own needs.

Three Pillars of Sustainability

- Environmental
- Economic
- Social

Sustainability takes an honest look at how a company’s business practices effect each of these pillars on an individual scale.

ACHIEVING SUSTAINABILITY

- Creating and maintaining a company that generates a profit
- Use available resources wisely (employees, materials, money)
- Reduce business’s effects on the environment
- Integrate and remain a successful part of the community and how it operates
- Manage, train and develop a conscientious staff

PERSONAL CARE MARKET + SUSTAINABILITY

The personal care and cosmetic industries directly affect all three pillars of sustainability

- Consume natural resources at an unsustainable rate
- Diminishing biodiversity

Initiatives, specifically by the European Trade and Cosmetic Association have been taken to reduce negative impacts and increase positive impacts

Evaluate operations to identify the social, economic and environmental impacts at every stage in the business value chain:

- Raw materials
- Product design + development
- Production
- Packaging
- Transport + retailing
- Use
- End of life

BENEFITS OF SUSTAINABILITY

- Reduce costs + sheds light on areas of wasted resources
- Inspires product development
- Maintain/ increase sales
- Fosters competitive position in the market + remain competitive in the future
- Employee Motivation + Employee Satisfaction = Increased Productivity + Improved Company Climate
- Boosts company reputation and community perception
- Promotes + nurtures supplier and retailer relationships
- Viewed as fiscally responsible leading to enhanced investments

A FACE-LIFT FOR THE STEM CELL

“The new age of anti-aging is how Cosmetic Design is describing plant stem cell technology, saying plant stem cell extracts are efficacy in a jar”

~ Eric Perrier/LVMH

The demand for stem cell technologies that promise potent anti-aging benefits has grown exponentially in the last decade

Cosmetic market has seen many incarnations of stem technology

- Plant stem cells
- Ingredients to activate endogenous adult stem cells
Plant stem cell technology facilitates the development of eco-friendly and sustainable cosmetic products. Technological advances allow cosmetic scientists to select, extract and improve natural molecules.
WHAT ARE STEM CELLS?

Non-specialized cells capable of self-renewal...

What this means...

Each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function

- Specialized cells define cell differentiation
STEM CELL DIFFERENTIATION

Cellular Plasticity:

- A specific characteristic of stem cells
- The cell’s ability to move from an undifferentiated state to a specific cell type

Types of Plasticity:

- **Pluripotent**-cells that can transform from a generic plant or animal cell into many different cell types
- **Totipotent**-cells that can transform into any cell type
Meristem Cells

- Pluripotent cells found in plants
- Have the ability to replicate beyond Hayflick’s Limit
- No ethical issues surround the use of plant stem cells (in contrast to embryonic stem cells)
- Currently two approaches to stem cells:
  - Stimulation of adult stem cell proliferation
  - The use of plant stem cells
Meristemic Cell Technology in Cosmetics

- Most popular form of stem cell technology
- Said to slow aging by defending against extrinsic stress
- This technology uses non-differentiated cells from simple cell extracts
- Real problem for “anti-aging” cosmetic products...
  - No specific activity
  - No cosmetic benefits
The Green Approach: Plant Cell Cultures

- Molecules of interest are highly concentrated in the meristem cells (buds)
- In culture, all cells are dedifferentiated cells with meristematic character
- Only requires a small amount of plant tissue to find active substance
- Completely eco-friendly, protects biodiversity, no harvesting wild plants or crops
- Full access to natural substances plus a higher presence of key plant molecules
- Numerous plants can be grown in culture in the presence of bacteria and yeast to enhance the production of secondary metabolites
A NEW APPROACH: THE TRUE PATH TO SUSTAINABILITY

A sustainable and eco-friendly product line

• The use of biotic stress induces secondary metabolites:
  ➢ Allows for the sustainable production of extracts precisely standardized for specific actives

• The use of secondary metabolites:
  ❖ Essential for the plant to interact with its environment
  ❖ Allows for adaptation, defense & ultimately survival in less than ideal conditions
A NEW APPROACH: BRAND DIFFERENTIATION + SUSTAINABILITY

Biotic Stress

- Damage caused by living organisms prompts plant to excrete stress responses
  - Examples: Bacteria, Fungi, Viruses, & Insects

Abiotic Stress

- A negative impact on plants due to extreme environmental conditions and/or pathogenic stress
  - Examples: Extreme Heat/ Cold, Intense UV Radiation, Flood/ Drought, Salicylate Production
A NEW APPROACH: BRAND DIFFERENTIATION + SUSTAINABILITY

Efficacious + Eco-Sustainable Extracts

- Continued destruction of plants poses a major extinction threat

- **Secondary metabolites** have no fundamental role in the maintenance of a plant’s life process

- Isolating these organic compounds through solvent extraction for specific benefits

- Numerous plants can be grown in culture in the presence of bacteria and yeast to enhance the production of secondary metabolites

- Allows for the differentiation of plant stem cell through pathogenic stress
PHYTO-BIOTICS PRODUCT LINE

- Use biotic stress embodied by sustainable practice of co-culturing plant cells with *Leuconostoc* or *Pseudomonas sp.*

- This approach induces cellular differentiation, or finely customized materials, standardized for specific activity and cosmetic skin benefits

- Phyto-Biotics Product Line promotes brand differentiation & a means of including patented, potent and efficacious actives
  - Cosmetic benefit claims link to the product not the active
  - Avoid intellectual property infringement
PHYTO-BIOTICS PRODUCT LINE

Phyto-Biotics Acai®

• Code - 16587

• INCI Name – Euterpe Oleracea Fruit Extract

• INCI Status – Approved

• Suggested Use Levels – 1.0-10.0%

• Suggested Applications – Anti-aging, Anti-wrinkle, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism, Moisturizing, Decrease in Moisture Regression
Addresses:

- Intrinsic + Extrinsic Aging
- Dehydrated Skin

How:

- Antioxidant protection
- Increased ATP Synthesis
- Enhanced Cellular Metabolism
- Potent moisturizing benefits that continue to moisturize the skin even after application has ceased
Why is Acai of Interest?

- This tree is known for its ability to survive in an extremely hot environment
- Tree evolved by producing secondary metabolites to survive

What Secondary Metabolite Helps Acai Trees Survive?

- **Ferulic Acid (C_{10}H_{10}O_{4})**:
  - Found in the plants cell wall components
  - Readily forms a resonance stabilized phenoxy radical
  - Acts as a potent antioxidant & ROS scavenger
**EFFICACY TESTING + PHYTO-BIOTICS ACAI®**

*In-vivo Moisturization Assay*

**Protocol**

- DermaLab Corneometer was used to measure moisture levels on the volar forearms.
- Corneometer is an instrument that measures the amount of water within the skin.
- The presence of moisture in the skin improves conductance & results in higher readings than dry skin.
- Baseline readings were taken on day one of the study (T=0).

**Average Moisturization**

- Experimental (2.0% Phyto-Biotics Acai®) in Base Lotion
- Base Lotion Control
- Untreated Control

**Graph 1.** Average moisturization levels measured at each test site.
**In-vivo Moisturization Assay**

**Protocol**

- DermaLab Corneometer was used to measure moisture levels on the volar forearms.
- Corneometer is an instrument that measures the amount of water within the skin.
- The presence of moisture in the skin improves conductance & results in higher readings than dry skin.
- Baseline readings were taken on day one of the study.

**Graph 2.** Comparative moisture analysis between test sites
**In-vitro Ferulic Acid Assay**

Improvements in Ferulic Acid Content in Acai Co-Cultured with *Leuconostoc sp.*

**Protocol**
- Genetically uniform, shoot-based clonal lines of Acai were isolated & co-cultured
- Inoculated with *Leuconostoc sp.* for 30 days
- Ferulic acid was extracted from both inoculated & un-inoculated
- Absorbance was measured at 333nm
- Improvements of Ferulic Acid content in the co-cultured Acai palms compared to the control

**Graph 3.** Compared to the control, Ferulic Acid content increases when Acai is co-cultured with *Leuconostoc sp.*

**Purpose of study** was to evaluate the effect of dosage on skin protection using known antioxidant solutions.

- Skin is pretreated with 75, 150 & 250 mL of 15.0% Vitamin C, 1.0% Vitamin E and 0.5% Ferulic Acid.
- Skin is irradiated with solar-stimulated radiation at a MED.
- Sunburn protection is dose dependent.

**Graph 4.** Effects of dosage on photo-protection when using vitamins C, E and Ferulic Acid.

**Protocol**

- Purpose of this study was to compare photo-protection by topical antioxidant formulations.

- Skin was pretreated with vehicle, 0.5% Ferulic Acid, 15.0% Vitamin C + 1.0% Vitamin E, 15.0% Vitamin C + 1.0% Vitamin E + 0.5% Ferulic Acid.

- Skin is irradiated with solar-stimulated radiation 2x – 10x minimal erythema dose (MED) at 2 x MED intervals.

- Evaluations 1 day later.

**Graph 5.** Colorimeter measurements of photoprotection by antioxidant solutions.

**In-vitro Ferulic Acid Assay**

**Visual Erythema + Antioxidant Photo Protection by Antioxidant Solutions**

**Protocol**

- Purpose of this study was to compare visual and antioxidant photo-protection by topical antioxidant formulations

- Skin was pretreated with vehicle, 0.5% Ferulic Acid, 15.0% Vitamin C + 1.0% Vitamin E, 15.0% Vitamin C + 1.0% Vitamin E + 0.5% Ferulic Acid

- Skin is irradiated with solar-stimulated radiation 2x – 10x minimal erythema dose (MED) at 2 x MED intervals

- Evaluations 1 day later

**Graph 6.** Visual erythema + antioxidant photoprotection by antioxidant solutions

EFFICACY TESTING + PHYTO-BIOTICS ACAI®

In-vitro ORAC Assay

**Protocol**

- Trolox used as a positive control
- Solution were prepared at three concentration as a reference
- Fluorescent measurements were taken every two minutes for two hours
- Phyto-Biotics Acai® showed antioxidant activity at 0.025% concentrations

**Graph 5.** The results indicated that Ferulic Acid provides comparable antioxidant activity to Trolox.
EFFICACY TESTING + PHYTO-BIOTICS ACAI®

In-vitro IL-6 ELISA Assay

Graph 6. Decrease in IL-6 production when using Phyto-Biotics Acai® indicates a reduced inflammatory environment.

Protocol

- Human fibroblasts are allowed to grow to confluency in complete DMEM.
- 1.0%, 0.1% and 0.01% of Phyto-Biotics Acai® was used
- Incubated with fibroblasts for 24 hours
- Decrease in IL-6 production, indicates a reduced inflammatory environment
- Decrease signs of aging & reduce formation of lines & wrinkles
EFFICACY TESTING + PHYTO-BIOTICS ACAI®

In-vitro Cellular Viability Assay

Protocol

- Measures cell mediated cytotoxicity, cell proliferation & metabolic activity
- Human fibroblasts were seeded into a 96 well tissue culture with complete DMEM
- Concentrations tested were 1%, 0.1% & 0.01%
- The fibroblasts were incubated for 24 hours
- .01% Phyto-Biotics Acai® increased cell metabolism

Graph 7. Cellular metabolism of Phyto-Biotics Acai® treated in fibroblasts expressed in terms of percent control
Phyto-Biotics Acai®

- **Code** - 16587
- **INCI Name** – Euterpe Oleracea Fruit Extract
- **INCI Status** – Approved
- **Suggested Use Levels** – 1.0-10.0%
- **Suggested Applications** – Anti-aging, Anti-wrinkle, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism, Moisturizing, Decrease in Moisture Regression
PHYTO-BIOTICS PRODUCT LINE

Phyto-Biotics Perilla

• **Code** – 40600

• **INCI Name** – Perilla Frutescens Extract

• **INCI Status** – Approved

• **Suggested Use Levels** – 1.0-10.0%

• **Suggested Applications** – Anti-aging, Anti-wrinkle, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism, Increases Moisturization
**PHYTO-BIOTICS PERILLA**

**Addresses:**
- Intrinsic + Extrinsic Aging
- Dehydrated Skin

**How:**
- Antioxidant protection
- Increased ATP Synthesis
- Reduces Appearance of Wrinkles
- Enhanced Cellular Metabolism
- Potent moisturizing benefit
 PHYTO-BIOTICS PERILLA

Brand Distinction Using Perilla

- Of particular interest – *Perilla frutescens*
- This Chinese basil is a popular medicinal treatment
- Can flourish in the most desolate conditions
- Its secondary metabolites provide:
  - Potent antioxidant properties
  - Soothing properties
  - Protection from inflammation caused by free radicals
What Secondary Metabolite Protects Perilla?

**Rosmarinic Acid (C\textsubscript{18}H\textsubscript{16}O\textsubscript{6}):**

- Ester of caffeic acid & 3,4 dihydroxyphenyllactic acid
- Its four phenolic hydrogens contribute to its ability to control free radical oxidation
- Contains two catechol (1,2 dihydroxybenzene) rings, giving it a quality of polarity
- Thus can form intermolecular hydrogen bonds between the free hydrogen of its hydroxyl & its phenoxy radical
- Significantly improving its radical stability
EFFICACY + PHYTO-BIOTICS PERILLA

**In-vivo Moisturization Assay**

**Average Increase in Moisture Levels**

<table>
<thead>
<tr>
<th>Time</th>
<th>0.0</th>
<th>50.0</th>
<th>100.0</th>
<th>150.0</th>
<th>200.0</th>
<th>250.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 24 Hours</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>T = 1 Week</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
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<tr>
<td>T = 2 Weeks</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>T = 3 Weeks</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
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<tr>
<td>T = 4 Weeks</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

- 2.0% Phyto-Biotics Perilla in Base Lotion
- Base Lotion
- Untreated

**Graph 1.** Average moisturization measured at each site

**Protocol**

- DermaLab Corneometer was used to measure moisture levels on the volar forearms.
- Corneometer is an instrument that measures the amount of water within the skin.
- The presence of moisture in the skin improves conductance & results in higher readings than dry skin.
- Baseline readings were taken on day one of the study.
**Efficacy + Phyto-Biotics Perilla**

**In-vivo Moisturization Assay**

**Protocol**

- DermaLab Corneometer was used to measure moisture levels on the volar forearms
- Corneometer is an instrument that measures the amount of water within the skin
- The presence of moisture in the skin improves conductance & results in higher readings than dry skin
- Baseline readings were taken on day one of the study

**Graph 2.** Comparative moisture analysis between test sites

![Graph](image)
In-vitro Rosmarinic Assay

**Improvements in Rosmarinic Acid in *Perilla frutescens* Co-Cultured with *Pseudomonas sp.***

**Protocol**
- Genetically uniform, shoot-based clonal lines of *Perilla frutescens* were isolated & co-cultured
- Inoculated with *Pseudomonas, sp* for 30 days
- Rosmarinic acid was extracted from both inoculated & un-inoculated
- Absorbance was measured at 333nm
- Improvements of Rosmarinic Acid content in the *co-cultured Perilla frutescens* compared to the control

**Graph 3.** Improvements in Rosmarinic Acid in *Perilla frutescens* co-cultured with *Pseudomonas sp.*

To evaluate antioxidant effect of Rosmarinic Acid (RA) on cultured human skin melanoma cells injured by ROS.

Cytotoxicity & antioxidant effects were analyzed by an XTT assay after cells were treated with H₂O₂.

Rosmarinic Acid increased cell adhesion activity & DPPH-radical scavenging activity in cells treated with H₂O₂.

Rosmarinic Acid showed antioxidant effects on ROS, such as H₂O₂.

Efficacy + Phyto-Biotics Perilla

In-vitro ORAC Assay

**Protocol**

- Trolox used as a positive control
- Solution were prepared at three concentration as a reference
- Fluorescent measurements were taken every two minutes for two hours
- **Phyto-Biotics Perilla** showed antioxidant activity at 0.1% concentrations

**Graph 5.** The results indicated that Phyto-Biotics Perilla provides comparable antioxidant activity to Trolox.
**Protocol**

- Human fibroblasts are allowed to grow to confluency in complete DMEM
- 1.0%, 0.1% and 0.01% of Phyto-biotics Perilla was used
- Incubated with fibroblasts for 24 hours
- A decrease in IL-6 production, indicates a reduced inflammatory environment
- Decrease signs of aging & reduce formation of lines & wrinkles

**Graph 6.** Decrease in IL-6 production when using Phyto-Biotics Perilla indicates a reduced inflammatory environment.
In-vitro Cellular Viability Assay

**Protocol**

- Measures cell mediated cytotoxicity, cell proliferation & metabolic activity
- Human fibroblasts were seeded into a 96 well tissue culture with complete DMEM
- Fibroblasts were incubated for 24 hours
- 0.10% & 0.01% concentrations of Phyto-Biotics Perilla increased cell metabolism

**Graph 7.** Cellular metabolism of Phyto-Biotics Perilla treated in fibroblasts expressed in terms of percent control.
Phyto-Biotics Perilla

- **Code** – 40600

- **INCI Name** – Perilla Frutescens Extract

- **INCI Status** – Approved

- **Suggested Use Levels** – 1.0-10.0%

- **Suggested Applications** – Anti-aging, Anti-wrinkle, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism, Increases Moisturization
PHYTO-BIOTICS PRODUCT LINE

Phyto-Biotics Quercus

- **Code** – 16588
- **INCI Name** – Quercus Alba Bark Extract
- **INCI Status** – Approved
- **Suggested Use Levels** – 1.0-10.0%
- **Suggested Applications** – Anti-aging, Anti-wrinkle, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism, Increases Moisturization, Improves Skin Density
Addresses:

- Intrinsic + Extrinsic Aging
- Dehydrated Skin
- Sagging Skin + Wrinkles

How:

- Antioxidant Protection
- Enhances ATP Synthesis + Cellular Metabolism
- Improves Skin Density
- Potent Moisturizing Benefit
Quercus – The Herbal Soother

- *Quercus alba*, or White Oak bark, was traditionally used for its astringent & anti-inflammatory effects

- Has high constituents of phenolic compounds, tannins & quercin

- Potent astringent properties:
  - Helps absorb toxins
  - Sooth irritated/swollen skin
  - Can improve appearance of problem skin
What Secondary Metabolite Protects Quercus?

**Tannic Acid (C\textsubscript{72}H\textsubscript{52}O\textsubscript{46}):**
- Ester of caffeic acid & 3,4 dihydroxyphenyllactic acid
- Its four phenolic hydrogens contribute to its ability to control free radical oxidation
- Contains two catechol (1,2 dihydroxybenzene) rings, giving it a quality of polarity
- Thus can form intermolecular hydrogen bongs between the free hydrogen of its hydroxyl & its phenoxy radical
- Significantly improving its radical stability

\[
\text{MW}=1,701.19 \text{ g/mol}
\]
**EFFICACY + PHYTO-BIOTICS QUERCUS**

*In-vivo* Moisturization Assay

**Protocol**

- DermaLab Corneometer was used to measure moisture levels on the volar forearms

- Corneometer is an instrument that measures the amount of water within the skin

- The presence of moisture in the skin improves conductance & results in higher readings than dry skin

- Baseline readings were taken on day one of the study

**Graph 1.** Average moisturization levels measured at each test site.
**EFFICACY + PHYTO-BIOTICS QUERCUS**

*In-vivo Moisturization Assay*

**Graph 2.** Comparative moisture analysis between test sites

**Comparative Moisture Results Between Test Sites**

<table>
<thead>
<tr>
<th>Time</th>
<th>2.0% Phyto-Biotics Quercus + Base Lotion vs. Untreated</th>
<th>2.0% Phyto-Biotics Quercus + Base Lotion vs. Base Lotion</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 24 Hours</td>
<td>0.00</td>
<td>40.00</td>
</tr>
<tr>
<td>T = 1 Week</td>
<td>20.00</td>
<td>60.00</td>
</tr>
<tr>
<td>T = 2 Weeks</td>
<td>40.00</td>
<td>80.00</td>
</tr>
<tr>
<td>T = 3 Weeks</td>
<td>60.00</td>
<td>100.00</td>
</tr>
<tr>
<td>T = 4 Weeks</td>
<td>80.00</td>
<td>120.00</td>
</tr>
</tbody>
</table>

**Protocol**

- DermaLab Corneometer was used to measure moisture levels on the volar forearms
- Corneometer is an instrument that measures the amount of water within the skin
- The presence of moisture in the skin improves conductance & results in higher readings than dry skin
- Baseline readings were taken on day one of the study
Efficacy + Phyto-Biotics Quercus

In-vivo Skin Density Assay

**Average Results Per Test Site**

<table>
<thead>
<tr>
<th>Time (Weeks)</th>
<th>Experimental (2.0% Phyto-Biotics Quercus + Base Lotion)</th>
<th>Base Lotion</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 0</td>
<td>70.00</td>
<td>70.00</td>
<td>70.00</td>
</tr>
<tr>
<td>T = 1 Week</td>
<td>75.00</td>
<td>75.00</td>
<td>75.00</td>
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<tr>
<td>T = 2 Weeks</td>
<td>80.00</td>
<td>80.00</td>
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<tr>
<td>T = 3 Weeks</td>
<td>85.00</td>
<td>85.00</td>
<td>85.00</td>
</tr>
<tr>
<td>T = 4 Weeks</td>
<td>90.00</td>
<td>90.00</td>
<td>90.00</td>
</tr>
</tbody>
</table>

**Protocol**

- 10 M/F subjects, ages 23-45. High Resolution Ultrasound Skin imaging was used on the volar forearms

- The intensity of the signals are received refer to a color scale

- Dark colors represent areas of skin with low reflection-no or little change in skin density

- Bright colors represent areas with strong reflections or substantial change in skin density

**Graph 3.** Skin density measured via a high resolution ultra sound which reports collagen level at each test site.
**EFFICACY + PHYTO-BIOTICS QUERCUS**

**In-vivo Skin Density Assay**

Collagen Ultrasound
Experimental vs. Base Lotion Treatment

**Protocol**

- 10 M/F subjects, ages 23-45. High Resolution Ultrasound Skin imaging was used on the volar forearms.

- The intensity of the signals are received refer to a color scale.

- Dark colors represent areas of skin with low reflection-no or little change in skin density.

- Bright colors represent areas with strong reflections or substantial change in skin density.

**Graph 4.** Percent Difference between test site collagen levels over 4 weeks.
**EFFICACY + PHYTO-BIOTICS QUERCUS**

**In-vitro Tannic Acid Assay**

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

**Protocol**

- Genetically uniform, shoot-based clonal lines of *Quercus alba* were isolated & co-cultured
- Inoculated with *Leuconostoc, sp* for 30 days
- Tannic acid was extracted from both inoculated & un-inoculated
- Absorbance was measured at 333nm
- Improvements of Tannic Acid content in the *co-cultured Quercus alba* compared to the control

**Graph 5.** Improvements in Tannic Acid in *Quercus alba* co-cultured with *Leuconostoc sp.*
Graph 6. The results indicated that Tannic Acid provides comparable antioxidant activity to Trolox.

**In-vitro ORAC Assay**

**Protocol**

- Trolox used as a positive control
- Solution were prepared at three concentration as a reference
- Fluorescent measurements were taken every two minutes for two hours
- **Phyto-Biotics Quercus** showed antioxidant activity at 0.5% concentrations
**EFFICACY + PHYTO-BIOTICS QUERCUS**

*In-vitro IL-6 Elisa Assay*

- **Graph 7.** Decrease in IL-6 production when using Phyto-Biotics Quercus indicates a reduced inflammatory environment.

**Protocol**

- Human fibroblasts are allowed to grow to confluency in complete DMEM
- 0.1% and 0.01% of Phyto-Biotics Quercus was used
- Incubated with fibroblasts for 24 hours
- Decrease in IL-6 production, indicates a reduced inflammatory environment
- Decrease signs of aging & reduce formation of lines & wrinkles
**Efficacy + Phyto-Biotics Quercus**

**In-vitro Cellular Viability Assay**

**Protocol**
- Measures cell mediated cytotoxicity, cell proliferation & metabolic activity
- Human fibroblasts were seeded into a 96 well tissue culture with complete DMEM
- Concentrations tested were 0.01%
- The fibroblasts were incubated for 24 hours
- **Phyto-Biotics Quercus** at a concentration of 0.01% increased cell metabolism

**Graph 8.** Cellular metabolism of Phyto-Biotics Quercus treated in fibroblasts expressed in terms of percent control.
PHYTO-BIOTICS PRODUCT LINE

Phyto-Biotics Quercus

- **Code** – 16588
- **INCI Name** – Quercus Alba Bark Extract
- **INCI Status** – Approved
- **Suggested Use Levels** – 1.0-10.0%
- **Suggested Applications** – Anti-aging, Anti-wrinkle, Soothing, Antioxidant, ATP Synthesis, Increases Cellular Metabolism, Increases Moisturization, Improves Skin Density
REFERENCES


EFFICACY + PHYTO-BIOTICS PERILLA

THANK YOU
For more information – Visit our website!
www.activeconceptsllc.com