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Tomorrow's Vision... *Today!*®

ACB Willow Bark Extract 20%
Efficacy Data

Code: 20200
INCI Name: Salix Nigra (Willow) Bark Extract
CAS #: 84650-64-6
EINECS #: 283-522-3

Type of Study	Results
Cellular Renewal Assay	**ACB Willow Bark Extract 20%** was evaluated for its ability to accelerate cell renewal by means of a traditional Dansyl Chloride protocol. The results indicate that **ACB Willow Bark Extract 20%** is capable of increasing cellular renewal by 27% when compared to the untreated biological control.
COX II Inhibition	As shown in figure 1, **ACB Willow Bark Extract 20%**was able to inhibit COX-II production. This decrease in COX-II production indicates a reduced inflammatory environment which can decrease the signs of aging and photodamage. For these reasons, we can assume **ACB Willow Bark Extract 20%** is suitable for cosmetic applications designed to provide anti-aging and anti-inflammatory properties.
Ultrasound Skin Imaging	As evidenced in a 4 week efficacy study of **ACB Willow Bark Extract 20%** on skin, skin density was improved by 33.59% after 24 hours and by 44.42% after 4 weeks when compared to the untreated control. When compared to the base cream **ACB Willow Bark Extract 20%** improved skin density by 36.72% after 24 hours and after 4 weeks **ACB Willow Bark Extract 20% Extract** improved density by 32.41%. Results indicate that **ACB Willow Bark Extract 20%** is capable of improving skin density when compared to both the untreated control as well as the base
IL-6 Elisa	As shown in figure 1, **ACB Willow Bark Extract 20%** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **ACB Willow Bark Extract 20%**appears to be dose dependent. 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.

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	Willow Bark Extract is suitable for cosmetic applications designed to provide soothing and anti-aging properties.
ORAC Assay	As shown in figure 1, **ACB Willow Bark Extract 20%** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **ACB Willow Bark Extract 20%** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent. **ACB Willow Bark Extract 20%** was designed for problem skin with exfoliation and antimicrobial properties.
Challenge Test	**ACB Willow Bark Extract 20%** was combined with water and EDTA to obtain a 3% final concentration. The following titers of microorganisms were added separately to these solutions, and quality control testing was done on the following days. Each 28-day test was repeated 3 times consecutively on the same test solution (for a total of 84 days**)**

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Cellular Renewal Assay

Tradename: ABS Willow Bark Extract

Code: 10200

Test Request Form #: 1111

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Abstract

ABS Willow Bark Extract was evaluated for its ability to accelerate cell renewal by means of a traditional Dansyl Chloride protocol.

Methods & Materials

A 5% Dansyl Chloride was prepared by dispersing Dansyl Chloride 95% (Sigma) in petrolatum. Approximately 0.2 g of the ointment was applied to three 2cm x 2cm locations on the volar forearm of 12 (M/F) subjects between the ages of 19 and 43. The material was allowed to remain in place for 24 hours at which time any excess ointment was removed.

Two products were tested, with the remaining untreated site serving as the biological control. The products were applied in a randomized fashion. Approximately 50 μl of product was applied to the appropriate test site once per day. The sites were then examined daily under ultraviolet light (SL-3660 Long Wave Ultra Violet, Black Light Eastern Corp., Westbury, Long Island, NY) for fluorescence. The test was continued until no fluorescence was detectable at any site. The values listed reflect the average time for each product.

Results:

Material	Concentration	Days	% Cell Renewal
Salicylic Acid	1%	17	23
Untreated Control	N/A	24	0
ABS Willow Bark Extract	2%	18	27

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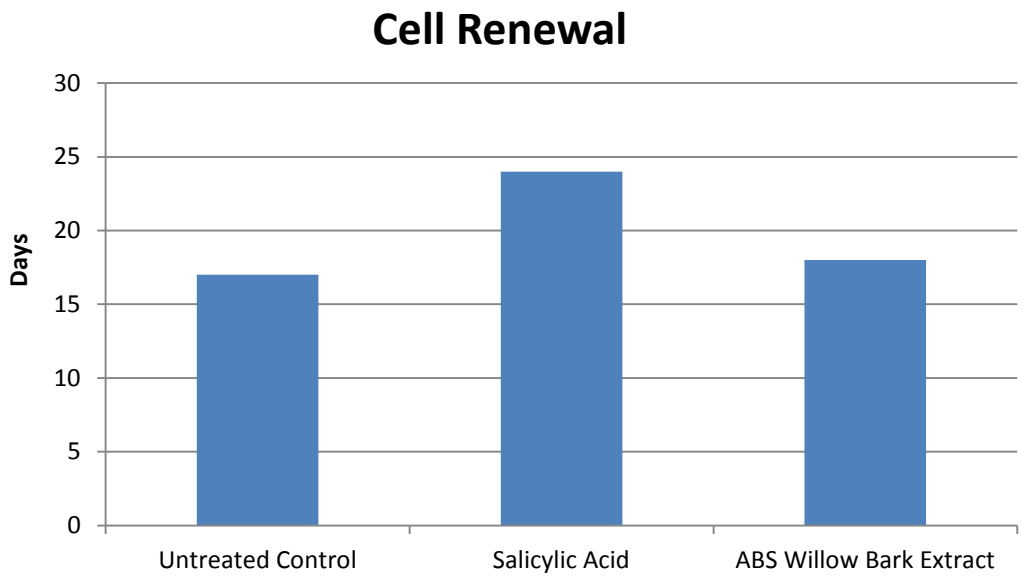

Discussion

The results indicate that **ABS Willow Bark Extract** is capable of increasing cellular renewal by 27% when compared to the untreated biological control.

COX-II Inhibition Assay Analysis

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Tradename: ABS Willow Bark Extract

Code: 10200

CAS #: 84650-64-6

Test Request Form #: 1037

Lot #: 36108

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
COX-II Inhibition Assay

Introduction

Cyclooxygenase (COX, also called Prostaglandin H Synthase or PGHS) is a bifunctional enzyme exhibiting both COX and peroxidase activities. The COX component converts arachidonic acids to hydroperoxy endoperoxide (PGG_2), and the peroxide component reduces endoperoxide to the corresponding alcohol (PGH_2), the precursor of prostaglandins (PGs), thromboxanes, and prostacyclins. It is well established that there are two isoforms of COX. COX-I is constitutively expressed in a variety of cell types and is involved in normal cellular homeostasis. Stimuli, such as phorbol esters, lipopolysaccharides, and cytokines lead to the induced expression of the second isoform, COX-II. COX-II is responsible for the biosynthesis of inflammatory mediators. COX-II also has a known role in aging due to increased expression seen in aged and photodamaged skin. The ability of a topical active ingredient to decrease COX-II expression is a beneficial anti-aging weapon.

This COX-II Inhibition Assay was conducted to assess the changes in COX-II levels in **ABS Willow Bark Extract**-treated *in vitro* cultured HaCat Keratinocytes and **ABS Willow Bark Extract** directly.

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Assay Principle

The COX-II Inhibitor Screening Assay directly measures Prostaglandin F2α ($PGF_{2\alpha}$) by stannous chloride ($SnCl_2$) reduction of COX-derived PGH_2 produced in the COX reaction. Stannous chloride is used to reduce PGH_2 to a more stable prostaglandin, $PGH_{2\alpha}$. The $PGF_{2\alpha}$ is quantified via enzyme immunoassay (EIA) which binds all major PG compounds using a specific antiserum. This assay is based on the competition between PGs and PG-acetylcholinesterase (AChE) conjugate for binding with antiserum-PG. The amount of PG-AChE conjugate will be held constant while the concentration of PGs varies depending on the sample, therefore the concentration of sample PGs in the well will be inversely proportional to the PG in the well. Ellman's Reagent is used to visualize the reaction of PG-AChE conjugate bound to antiserum-PG, which has a distinct yellow color that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve, along with manufacturer specified calculations provides a reference from the OD readings for the amount of COX-II in each sample.

Materials

A. Kit:	COX-II Inhibitor Screen Assay Kit (Cayman Chemical; 560131)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes
D. Cell Line:	HaCat Keratinocytes (AddexBio; T0020001)
E. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Amphotericin (45pg/mL)
F. Culture Plate:	Falcon flat bottom 12-well tissue culture treated plates
G. Reagents:	Dexamethasone (10µM)
H. Other:	Sterile disposable pipette tips; wash bottles

Methods

HaCat Keratinocytes were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 1% concentration of **ABS Willow Bark Extract** was added to complete DMEM and incubated with keratinocytes for 72 hours. Dexamethasone (DEX) was used as a positive control due to its known ability to inhibit COX-II. The cell culture supernatant is used as the experimental sample in the assay. Additionally, this assay allows for our experimental COX-II inhibitor samples to be added directly to the ELISA. Testing our experimental COX-II inhibitors with both methods gives us a better idea of how the material functions in and out of a biologic setting. **ABS Willow Bark Extract** was tested at the highest concentration possible because cell viability does not have to factored in.

The COX reaction is carried out using Human Recombinant COX-II, Heme to activate the peroxidase activity of COX-II, Arachidonic Acid as the reaction substrate, Hydrochloric Acid to stop the reaction, and Stannous Chloride to reduce PGF_2 to $PGF_{2\alpha}$. All of these reagents are provided in the manufacture's kit and used with our sample cell culture supernatants, the sample directly, and dexamethasone to obtain the solution to be used in the COX-II quantification EIA.

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Prostaglandin standards were prepared in concentrations ranging from 2000pg/mL to 0pg/mL. 100µL of EIA Buffer, which is provided in kit from manufacturer, was added to the 'Non-Specific Binding' (NSB) wells to account for non-immunologic binding of PG-AChE conjugate. 50µL of EIA Buffer was added to the 'Maximum Binding' wells. 50µL of each sample, standard, and internal assay controls were added in duplicate to the microtitre plate. Next, 50µL of PG-AChE conjugate was added to all wells. Finally, 50µL of the PG Antiserum was added to all wells except the NSB wells. The microtitre plate was incubated at room temperature for 18 hours on an orbital shaker.

For development of the plate, all well were rinsed with EIA Wash Buffer, proved in the kit from the manufacturer. 200µL of Ellman's Reagent, also provided in the kit, was added to each well. 5µL of PG-AChE conjugate was added to a 'Total Activity' well to show maximum activity of the Ellman's Reagent. The reaction developed for 1 hour at room temperature on an orbital shaker. The absorbance was measured at 412nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a 4-parameter logistic curve fit. The COX-II concentration of **ABS Willow Bark Extract** was determined by extrapolation from the standard curve and expressed in terms of percent inhibition.

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Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

COX-II production percent inhibition is calculated by the following formula:

$$\boldsymbol{Percent}\ (\%)\ \boldsymbol{Inhibition}$$

$$= \frac{(\%Bound/Maximum\ Bound)_{COX-II\ 100\%\ Activity} - (\%Bound/Maximum\ Bound)_{Sample}}{(\%Bound/Maximum\ Bound)_{COX-II\ 100\%\ Activity}} \times 100$$

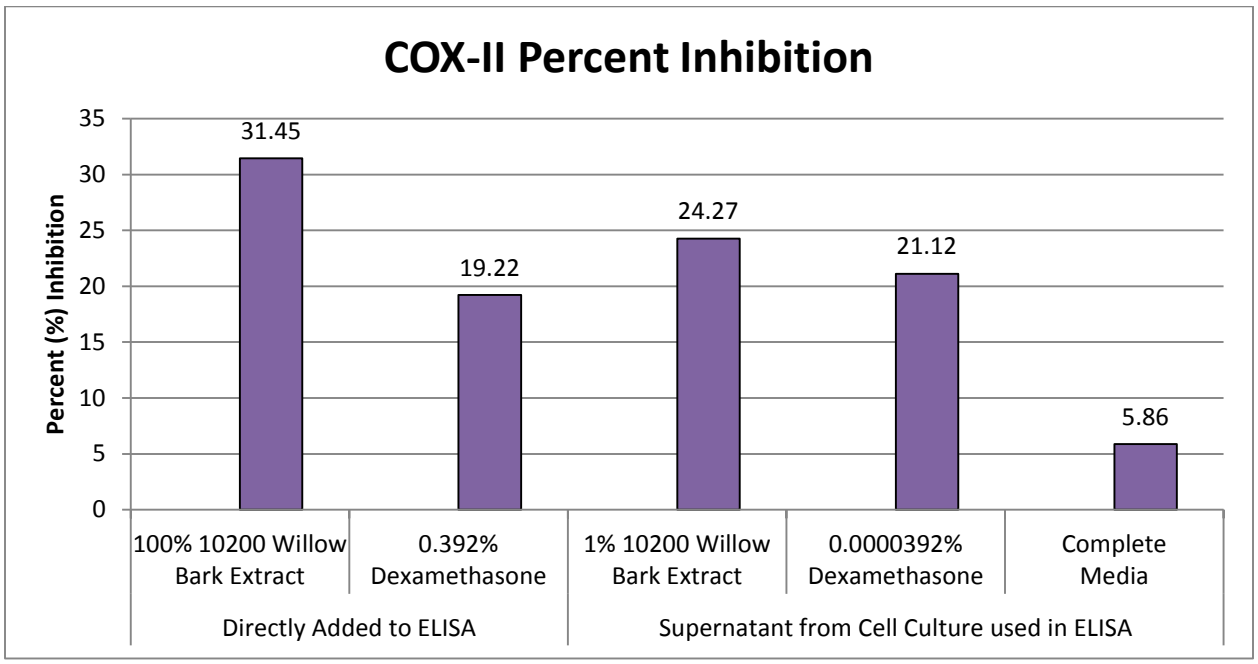

Figure 1: COX-II Percent Inhibition

Discussion

As shown in figure 1, **ABS Willow Bark Extract** was able to inhibit COX-II production. This decrease in COX-II production indicates a reduced inflammatory environment which can decrease the signs of aging and photodamage. For these reasons, we can assume **ABS Willow Bark Extract** is suitable for cosmetic applications designed to provide anti-aging and anti-inflammatory properties.

High Resolution Ultrasound Skin Imaging Assay

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Tradename: ABS Willow Bark Extract

Code: 10200

CAS #: 84650-64-6

Test Request Form #: 1112

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
High Resolution Ultrasound Skin-Imaging Assay

Introduction

An *in-vivo* study was conducted over a period of four weeks to evaluate the effect on skin density of **ABS Willow Bark Extract.** 10 M/F subjects between the ages of 23-45 participated in the study. Results indicate that this material is capable of significantly improving skin density compared to the control.

Materials

A. Equipment: DermaLab Skin Combo (Ultrasound Probe)

Methods

High Resolution Ultrasound Skin imaging is based on measuring the acoustic response after an acoustic pulse is sent into the skin. The energy of the acoustic pulse is low and will not affect the skin in any way. When the acoustic pulse is emitted and hits different areas of the skin, part of the pulse will be reflected and part will be transmitted further into the skin. The reflected signal travels back and is picked up by the ultrasound transducer. After processing the signal, a cross-sectional image appears on the screen. This image represents an intensity, or amplitude, analysis of the signals.

The intensity of the signals that are received refer to a color scale. Dark colors represent areas of the skin with low reflection. This means that there are no changes or very small changes in density between the structures in the skin. Bright colors represent areas with strong reflections, signifying substantial changes in density between structures.

10 volunteers M/F between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. The DermaLab ultrasound probe was used to determine the skin density of the subject's volar forearms. Baseline elasticity readings were taken on day one of the study.

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Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken immediately after application of test materials and then weekly for 4 weeks. The test material consisted of 2.0% **ABS Willow Bark Extract** in a base lotion.

For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

Results

ABS Willow Bark Extract showed improvements in skin density at a 2.0% concentration. Please note, each value is an average of three consecutive readings per test site.

Individual Raw Data:

	T = 24	1 week	2 weeks	3 weeks	4 weeks
Subject 1-Test	60	68	75	54	67
Untreated Control	53	54	50	42	45
Base Lotion Control	47	44	45	42	41
Subject 2-Test	100	98	93	99	82
Untreated Control	51	60	65	65	68
Base Lotion Control	65	69	89	86	75
Subject 3-Test	84	97	81	82	0
Untreated Control	71	67	68	64	0
Base Lotion Control	70	59	62	79	0
Subject 4-Test	84	93	85	77	98
Untreated Control	72	59	54	62	60
Base Lotion Control	50	70	74	52	67
Subject 5-Test	76	82	89	94	96
Untreated Control	46	53	43	50	58
Base Lotion Control	47	53	42	59	59
Subject 6-Test	85	79	92	69	0
Untreated Control	40	46	56	38	0
Base Lotion Control	44	59	46	43	0

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	T = 24	1 week	2 weeks	3 weeks	4 weeks
Subject 7-Test	66	70	63	45	66
Untreated Control	60	60	58	49	45
Base Lotion Control	63	51	58	65	61
Subject 8-Test	0	84	82	64	62
Untreated Control	0	51	50	52	42
Base Lotion Control	0	58	48	45	47
Subject 9-Test	70	69	72	61	69
Untreated Control	55	43	49	29	40
Base Lotion Control	54	50	55	34	52
Subject 10-Test	75	92	88	83	81
Untreated Control	76	69	74	68	72
Base Lotion Control	72	70	66	69	67
# of Subjects	9	10	10	10	8

Results of Group:

	t = 24	1 week	2 week	3 week	4 week
Experimental (2.0% ABS Willow Bark Extract in Base Lotion)	77.8	83.2	82.0	72.8	77.6
Untreated	58.2	56.2	56.7	51.9	53.8
Base Lotion Control	56.9	58.3	58.5	57.4	58.6
	t = 24	**1 week**	**2 week**	**3 week**	**4 week**
Experimental vs. Untreated Control	33.59%	48.04%	44.62%	40.27%	44.42%
Base Lotion vs. Untreated Control	-2.29%	3.74%	3.17%	10.60%	9.07%
Experimental vs. Base Lotion	36.72%	42.71%	40.17%	26.83%	32.41%

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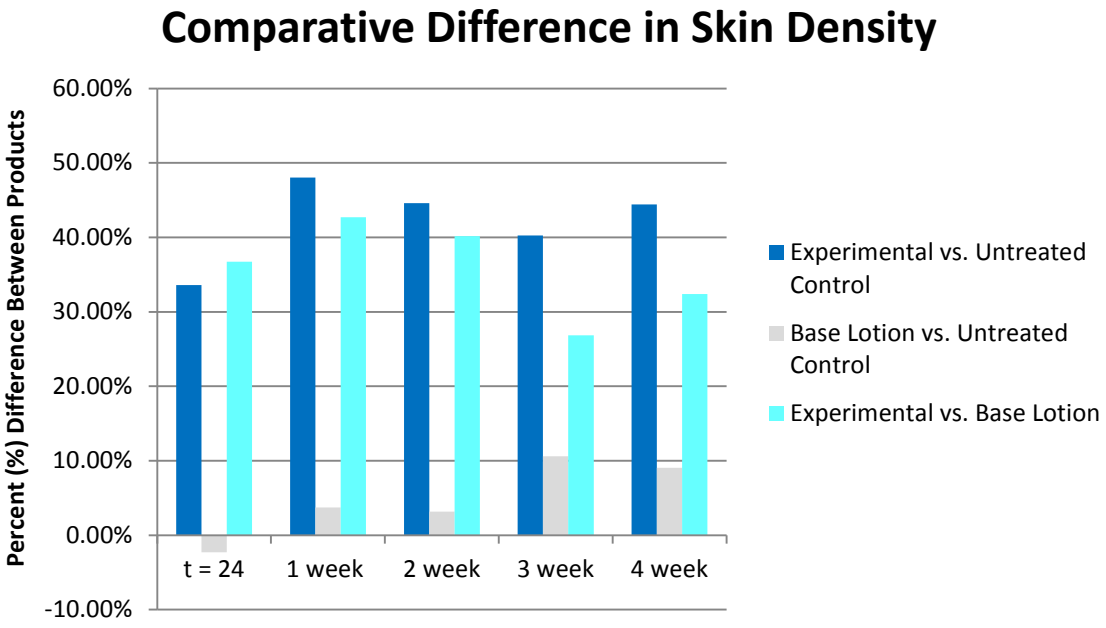

Figure 1: High Resolution Ultrasound Skin Imaging Results

Discussion

As evidenced in a 4 week efficacy study of **ABS Willow Bark Extract** on skin, skin density was improved by 33.59% after 24 hours and by 44.42% after 4 weeks when compared to the untreated control. When compared to the base cream **ABS Willow Bark Extract** improved skin density by 36.72% after 24 hours and after 4 weeks **ABS Willow Bark Extract** improved density by 32.41%. Results indicate that **ABS Willow Bark Extract** is capable of improving skin density when compared to both the untreated control as well as the base lotion.

ABS Willow Bark Extract has a strong positive effect on skin's density when used at recommended use levels.

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IL-6 ELISA Analysis

Tradename: ABS Willow Bark Extract

Code: 10200

CAS #: 84650-64-6

Test Request Form #: 1113

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Interleukin (IL)-6 Enzyme-Linked Immunosorbent Assay (ELISA)

Introduction

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-κB) 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.

Interleukin-6 ELISA was conducted to assess the changes in IL-6 levels in **ABS Willow Bark Extract**-treated *in vitro* cultured human dermal fibroblasts.

Assay Principle

This ELISA utilizes a colorimetric reaction employing antibodies with antigen specificity to human IL-6. Monoclonal antibodies specific for IL-6 epitopes are coated on a microtiter plate. In positive samples, IL-6 will bind to these antibodies and are tagged a second time with another IL-6-specific antibody labeled with horseradish peroxidase (HRP). The addition of the chromagen solution, containing 3,3',5,5'-tetramethylbenzidine, provides the colorimetric reaction with HRP that is quantitated through optical density (OD) readings on a microplate spectrometer. The standard curve provides a reference from the OD readings for the amount of collagen in each sample.

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Materials

A. Kit:	IL-6 ELISA Kit (Biosource; KAC1261)
B. Incubation Conditions:	37°C at 5% CO_2 and 95% relative humidity (RH)
C. Equipment:	Forma humidified incubator; ESCO biosafety laminar flow hood; Microplate Reader; Pipettes
D. Cell Line:	Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers:	Dulbecco's Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS); Amphotericin (45pg/mL)
F. Culture Plate:	Falcon flat bottom 12-well tissue culture treated plates
G. Reagents:	Lipopolysaccharide (LPS) (1µg/mL); Solution A (human plasma with preservatives); Solution B (buffer with preservative)
H. Other:	Sterile disposable pipette tips; wash bottles

Methods

Human dermal fibroblasts were seeded into 12-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, 0.01% concentrations of **ABS Willow Bark Extract** were added to complete DMEM containing 1µg/mL LPS and incubated with fibroblasts for 24 hours. Complete media containing 1µg/mL LPS was used as the positive controls and complete DMEM was used a negative control.

Standards were prepared in concentrations ranging from 0pg/mL to 2476pg/mL. 50µL of Solution B was added to wells for standards and assay controls and 50µL of Solution A was added to experiment wells. 100µL of standards, controls, and samples were added to appropriate wells. After a one hour incubation at room temperature and washing, 50µL Solution A and 100µL anti-IL-6 conjugate was added to all wells. Following a one hour incubation and washing, 100 µL chromagen solution was added for the colorimetric reaction. One-hundred µL stop solution was added to stop the reaction after 15 minutes. The optical density was read at 450nm on the Synergy HT Microplate Reader.

A standard curve was created by reducing the data and generating a linear curve fit. The IL-6 concentration of **ABS Willow Bark Extract** treated-fibroblasts was determined by extrapolation from the standard curve and expressed in pg/mL.

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Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

ABS Willow Bark Extract, at a concentration of 1% was able to decrease IL-6 production compared to our positive control.

IL-6 levels are expressed by the following formula:

$$= Average_{IL-6\ Concentrations} \times Dilution\ Factor$$

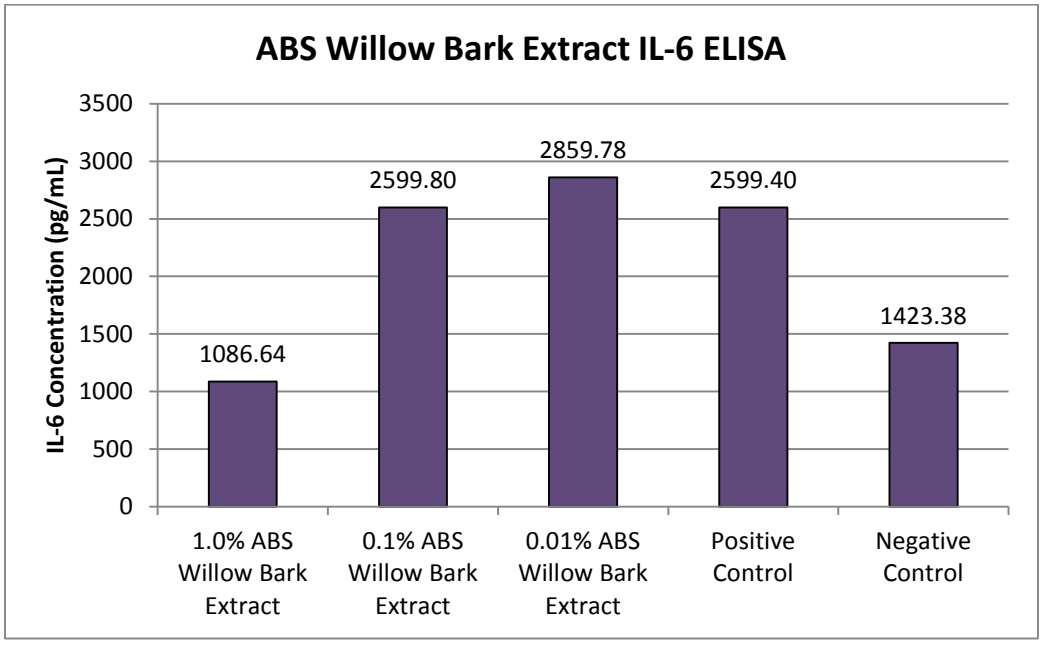

Figure 1: ABS Wilow Bark Extract-treated fibroblasts IL-6 concentrations

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IL-6 production percent decrease is calculated by the following formula:

$$= \frac{Positive\ Control_{\ Avg.Concentration} - Sample_{\ Avg.Concentration}}{Positive\ Control_{\ Avg.Concentration}} \times 100$$

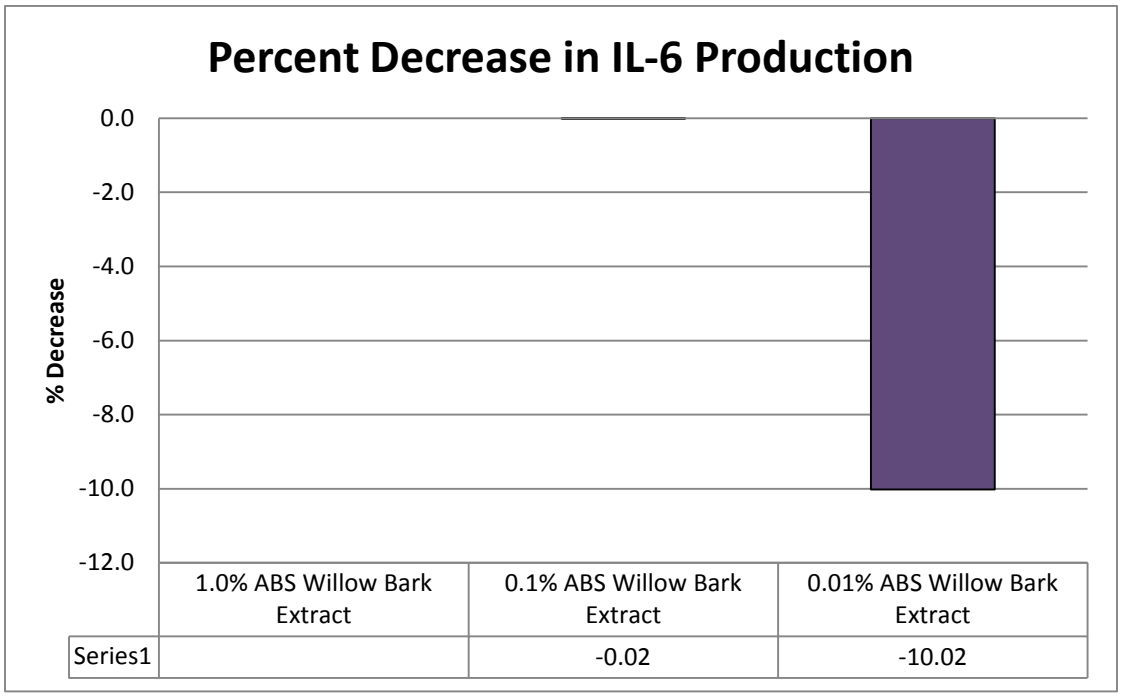

Figure 2: Percent decrease in IL-6 production compared to positive control

Discussion

As shown in figure 1, **ABS Willow Bark Extract** exhibited anti-inflammatory effects on LPS-treated fibroblasts. As expected, the changes in IL-6 production using **ABS Willow Bark Extract** appears to be dose dependent. 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. For these reasons, we can assume **ABS Willow Bark Extract** is suitable for cosmetic applications designed to provide soothing and anti-aging properties.

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: ABS Willow Bark Extract

Code: 10200

CAS #: 84650-64-6

Test Request Form #: 1114

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC)

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are detrimental to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **ABS Willow Bark Extract**.

Assay Principle

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.

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Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	75mM Potassium Phosphate (pH 7.4); Deionized H_2O
C. Reagents:	2,2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM)
D. Preparation:	Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.
E. Microtitre Plates:	Corning 96 Well Black Side/Clear Bottom Microplates

Methods

Solutions of **ABS Willow Bark Extract** and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37˚C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

$$AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow Where\ R\ is\ fluorescence\ reading$$

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample's Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents (µMTE), where 1 ORAC unit is equal to 1 µMTE.

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Results

ABS Willow Bark Extract began exhibiting antioxidant activity at a 0.0005% concentration.

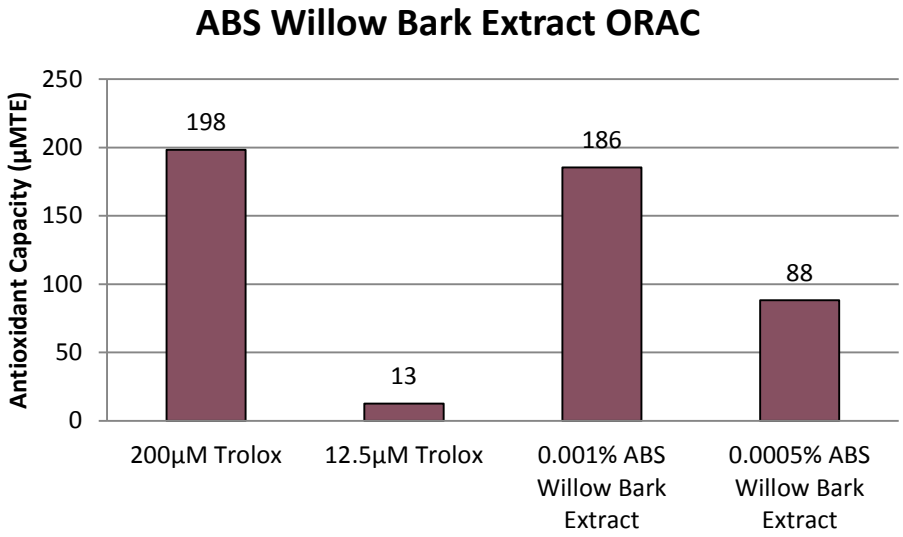

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **ABS Willow Bark Extract** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **ABS Willow Bark Extract** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

ABS Willow Bark Extract was designed for problem skin with exfoliation and antimicrobial properties. With the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic applications.

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ABS White Willow Bark Extract Powder

Code: 10229

Anti-microbial Challenge Test

ABS White Willow Bark Extract Powder was combined with water and EDTA to obtain a 3% final concentration. The following titers of microorganisms were added separately to these solutions, and quality control testing was done on the following days. Each 28-day test was repeated 3 times consecutively on the same test solution (for a total of 84 days).

Table 1. 3% ABS White Willow Bark Extract Powder						
	mold	yeast	E.coli	S.aureus	B.subtilis	P.aeruginosa
Initial	1.0×10^4	1.2×10^6	7.2×10^6	1.8×10^6	8.8×10^5	1.2×10^7
Day 3	<10	<10	<10	<10	<10	<10
Day 7	<10	<10	<10	<10	<10	<10
Day 14	<10	<10	<10	<10	<10	<10
Day 21	<10	<10	<10	<10	<10	<10
Day 28	<10	<10	<10	<10	<10	<10

Table 2. 3% ABS White Willow Bark Extract Powder						
	mold	yeast	E.coli	S.aureus	B.subtilis	P.aeruginosa
Day 28 Initial	1.0×10^4	1.2×10^6	7.2×10^6	1.8×10^6	8.8×10^5	1.2×10^7
Day 31	<10	<10	<10	<10	<10	<10
Day 35	<10	<10	<10	<10	<10	<10
Day 42	<10	<10	<10	<10	<10	<10
Day 49	<10	<10	<10	<10	<10	<10
Day 56	<10	<10	<10	<10	<10	<10

Table 3. 3% ABS White Willow Bark Extract Powder						
	mold	yeast	E.coli	S.aureus	B.subtilis	P.aeruginosa
Day 56 Initial	1.0×10^4	1.2×10^6	7.2×10^6	1.8×10^6	8.8×10^5	1.2×10^7
Day 59	<10	<10	<10	<10	<10	<10
Day 63	<10	<10	<10	<10	<10	<10
Day 70	<10	<10	<10	<10	<10	<10
Day 77	<10	<10	<10	<10	<10	<10
Day 84	<10	<10	<10	<10	<10	<10

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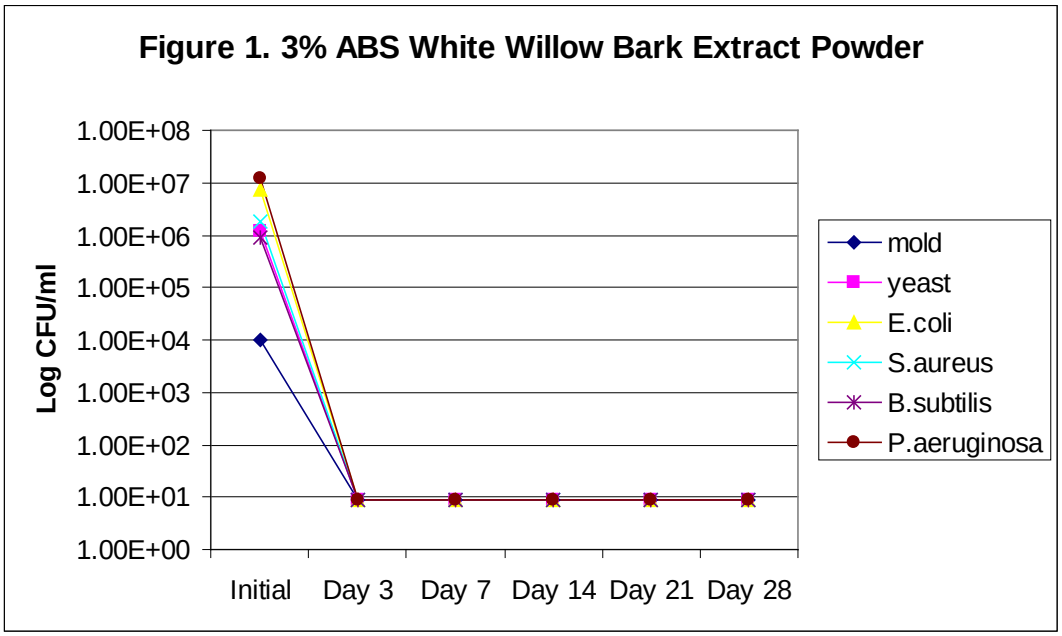

Figure 1. On Day 0, a **3% ABS White Willow Bark Extract Powder** solution was inoculated with the selected microorganisms.

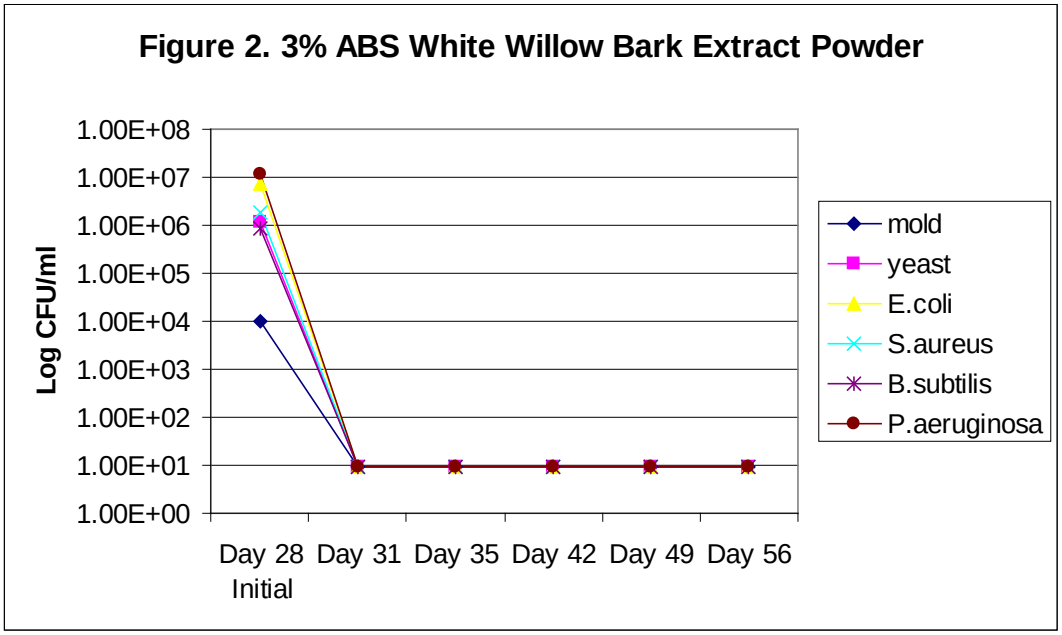

Figure 2. On Day 28, the same **3% ABS White Willow Bark Extract Powder** solution was re-inoculated with the selected microorganisms.

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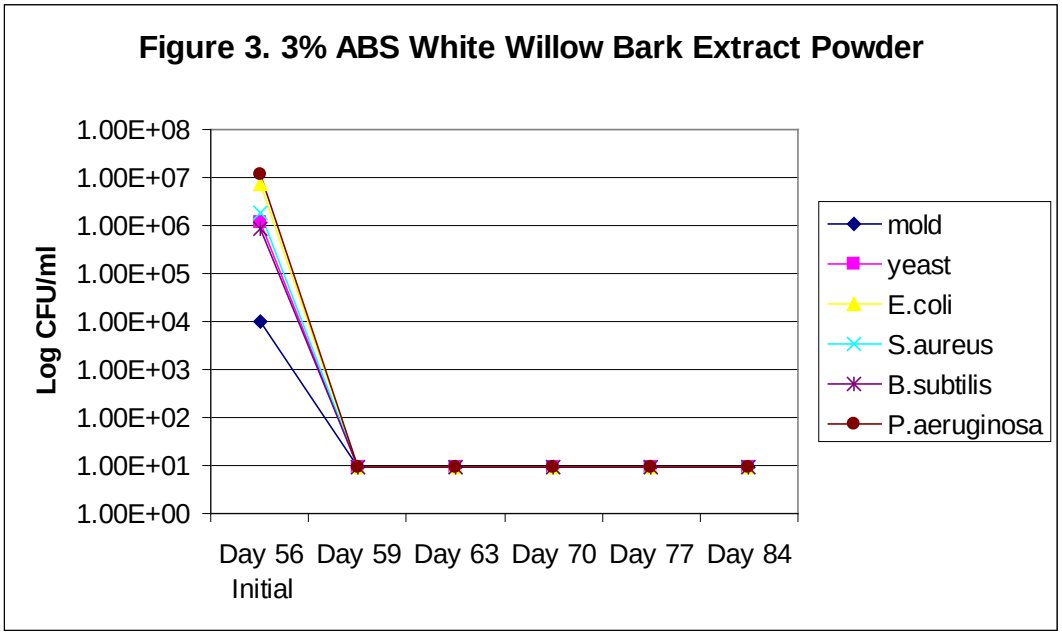

Figure 3. On Day 56, the same **3% ABS White Willow Bark Extract Powder** solution was re-inoculated with the selected microorganisms.