Active Concepts
Tomorrow's Vision... *Today!*®

ACB Yerba Santa Glycoprotein PF Efficacy Data

Code: 20342PF
INCI Name: Lactobacillus/Eriodictyon Californicum Ferment Filtrate
CAS #: 68990-14-7
EINECS #: 273-580-8

Type of Study	Results
Moisturization Study	The chart shows the results of the % moisturization at day 1 and day 30 of the study. The graph shows the results of the percent increase in moisturization comparing Aloe Vera and Yerba Santa Glycoprotein.
Cellular Viability Assay	In this study, **ACB Yerba Santa Glycoprotein PF** (code 20342PF) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of both 0.1% and 0.01% **ACB Yerba Santa Glycoprotein PF** (code 20342PF), nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **ACB Yerba Santa Glycoprotein PF** (code 20342PF) is not cytotoxic.
ORAC Assay	As shown in figure 1, **ACB Yerba Santa Glycoprotein PF** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **ACB Yerba Santa Glycoprotein PF** increased as the concentration increased. As a result we can assure that its ability to minimize oxidative stress is dose dependent. With the present study we can confirm that this unique ingredient is capable of providing antioxidant benefits when added to cosmetic applications.
PM 2.5 Inhibition	**ACB Yerba Santa Glycoprotein PF** was able to effectively prevent the accumulation of PM 2.5um sized particles on the skin.

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Yerba Santa Skin Moisturization Study

Specific Aim:

An In-Vivo clinical Twelve- (12) subject study was carried out over a thirty-day period to evaluate the long-term effects of the cosmetic raw material, Yerba Santa Glycoprotein on skin moisturization.

Materials:

Products:

Product 1: Blank Formulation Base (Water) + Aloe Vera 10X Gel (Control)
Product 2: Blank Formulation Base (Water) + 5% Yerba Santa Glycoprotein

Equipment:

Nova Impedance Meter

Methods:

Active Concepts utilized 12 female volunteers between the ages of 18 and 45, known to be free of any skin pathologies.

On day one of the study the moisturization levels of the volunteers' skin were measured via Nova Impedance Meter. This measurement was used as the subject's baseline.

We then divided the volunteers into two separate groups – Group 1 (6 volunteers) used Product 1 on the right side of the face. The left side of the face remained untreated. Group 2 (6 volunteers) used Product 2 on the right side of the face. The left side of the face remained untreated.

The twelve subjects followed the protocol for thirty days, applying the products once daily on the appropriate side of their face.

On day 30 Moisturization was once again assessed on the two halves of the face of each of the volunteers using the Nova Impedance Meter.

The amount of moisturization obtained after the 30 day study was evaluated and compared to the measurements taken on day one the study. Significant differences were found between the two groups of volunteers.

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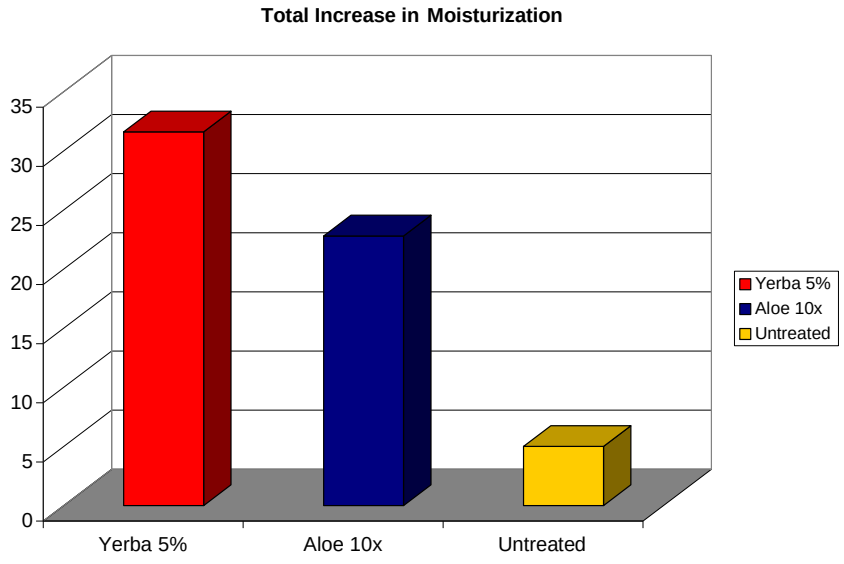
Results/Conclusion:

The following chart shows the results of the % moisturization at day 1 and day 30 of the study. The graph shows the results of the percent increase in moisturization comparing Aloe Vera and Yerba Santa Glycoprotein.

Group 1(Aloe)	
Day 1 Average % Moisturization	5.20%
Day 30 Average % Moisturization	28%
Group 2(Yerba)	
Day 1 Average % Moisturization	5.40%
Day 30 Average % Moisturization	37%



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Cellular Viability Assay Analysis

Tradename: ACB Yerba Santa Glycoprotein PF

Code: 20342PF

CAS #: 68990-14-7

Test Request Form #: 622

Lot #: 22061

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

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring 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 is ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of **ACB Yerba Santa Glycoprotein PF** to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.

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Materials

A.	**Kit:**	PrestoBlue™ Cell Viability Reagent (Invitrogen, A13261)
B.	**Incubation Conditions:**	37°C at 5% CO_2 and 95% relative humidity (RH)
C.	**Equipment:**	Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; 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)
F.	**Culture Plate:**	Falcon flat bottom 96-well tissue culture treated plates
G.	**Reagents:**	PrestoBlue™ reagent (10X)
H.	**Other:**	Sterile disposable pipette tips

Methods

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 **ACB Yerba Santa Glycoprotein PF** 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 90µL of cell culture media in culture wells and a fluorometric measurement was taken at 560nm for excitation and 590nm for emission.

Results

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

ACB Yerba Santa Glycoprotein PF did not exhibit significant effects on the cellular metabolism.

Cellular metabolism results are shown as mean fluorescence units (MFU) and expressed as percentage change, calculated by the below equation:

$$\text{Percent Change} = \frac{\text{Treated} - \text{Control}}{\text{Control}} \times 100$$

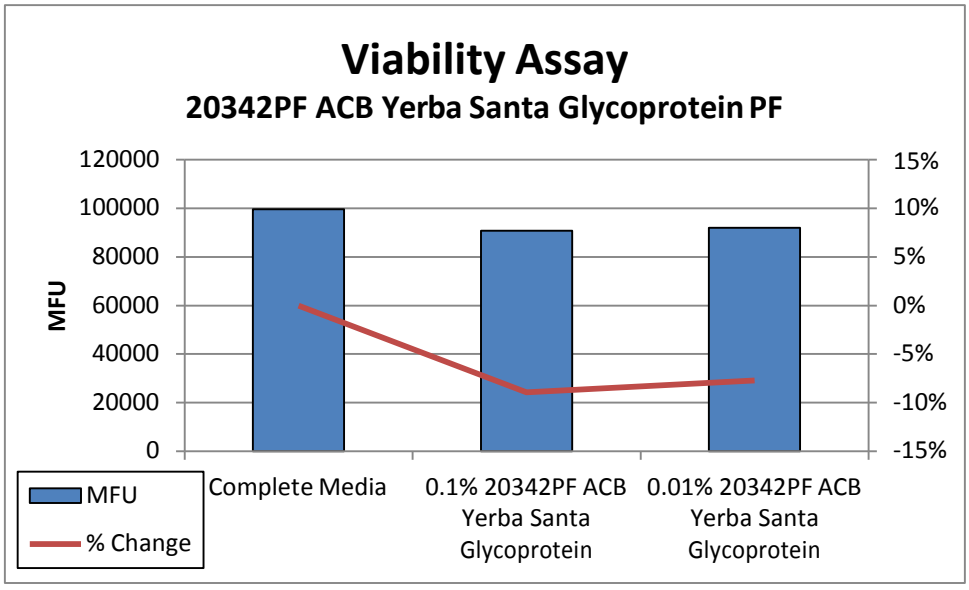

Figure 1: Cellular Metabolism of **ACB Yerba Santa Glycoprotein PF**-treated fibroblasts expressed in terms of percent of control.

Discussion

In this study, **ACB Yerba Santa Glycoprotein PF** (code 20342PF) was tested to evaluate its effects on the viability of normal human dermal fibroblasts (NDHF). At concentrations of both 0.1% and 0.01% **ACB Yerba Santa Glycoprotein PF** (code 20342PF), nor the preservatives contained therein exhibited any inhibition of cell viability. It can therefore be concluded that at normal use concentrations **ACB Yerba Santa Glycoprotein PF** (code 20342PF) is not cytotoxic.

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: ACB Yerba Santa Glycoprotein PF

Code: 20342PF

CAS #: 68990-14-7

Test Request Form #: 596

Lot #: 32061

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 **ACB Yerba Santa Glycoprotein PF**.

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 **ACB Yerba Santa Glycoprotein PF** 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:

$$AAAAAA = 0.5 + \frac{RR2}{RR1} + \frac{RR3}{RR1} + \frac{RR4}{RR1} + \cdots + \frac{RRRR}{RR1} \rightarrow WWheeeeee\ RR\ iiii\ fffffffeeeeiiffeeRRffee\ eeeerrrriiRRrr$$

$$NNeeNN\ AAAAAA = AAAAAA_{sssssssssss} - AAAAAA_{bbsssssbbbb}$$

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 1µMTE.

ORAC values are also calculated in Units/milliliter (U/mL). The equation used for the calculation is shown below:

$$OORRAAAA\ (AA/mmmm) = (50 \times DDiiffffNNiiffRR\ FFrrffNNffee) \times \left(\frac{AAAAAA_{SSSSSSSSSSS} - AAAAAA_{BBSSSSBBBB}}{AAAAAA_{TTTTTTSTTTT} - AAAAAA_{BBSSSSBBBB}} \right)$$

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Results

ACB Yerba Santa Glycoprotein PF began exhibiting antioxidant activity at a 0.05% concentration.

At concentrations exceeding 0.1%, the antioxidant activity is too intense to measure, resulting in over-saturation of the signal. This over-saturation produces results that exceed those of the standard curve. Therefore, lower concentrations were tested to ensure an accurate result.

The ORAC value expressed in U/mL for 0.1% **ACB Agave HSP** is 39382.9.

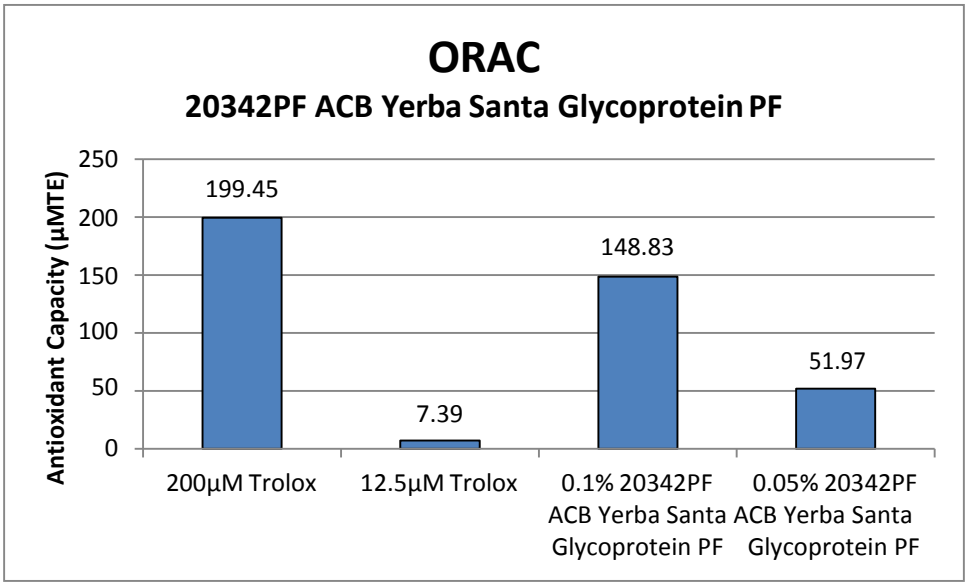

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **ACB Yerba Santa Glycoprotein PF** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **ACB Yerba Santa Glycoprotein PF** increased as the concentration increased. As a result we can assure that its ability to minimize oxidative stress is dose dependent. With the present study we can confirm that this unique ingredient is capable of providing antioxidant benefits when added to cosmetic applications.

Anti-Pollution Assay Analysis

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Tradename: ACB Yerba Santa Glycoprotein PF

Code: 20342PF

CAS #: 68990-14-7

Test Request Form #: 1688

Lot #: NC160112-C

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

Test Performed:
Pollution Protection Assay

Introduction

The role of pollution in the appearance of the premature wrinkles and age spots has because a new frontier in antiaging active ingredients. While we have known about the harmful effects of pollution on our health for years, new research indicates air pollution plays a detrimental role in extrinsic aging. Carbon and metal micro particles found in polluted air embed in the dermis causing oxidative stress, initiating the inflammatory cascade leading to the breakdown on the collagen, elastin, and other structural components in the skin. Additionally, polyaromatic hydrocarbons overstimulate the aryl hydrocarbon receptors on keratinocytes and melanocytes resulting in the hyperpigmentation and the appearance of age spots. Providing a physical barrier will prevent embedment of carbon particles, thus reducing the signs extrinsic aging.

Our pollution protection assay was conducted to assess the ability of **ACB Yerba Santa Glycoprotein PF** to provide immediate protection from carbon air pollution.

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Materials

A. Equipment:	Dissecting microscope; Digital camera; Pipettes
B. Reagents:	Micronized activated charcoal; Cetaphil Moisturizing for All Skin Types
C. Other:	Disposable pipette tips; wash bottles

Methods

Volunteers, male and female, between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. All subjects were asked to apply 2 mg of each test material, experimental, control, and untreated on their volar forearms. Lotions were allowed to dry completely before the addition of 5 mg of micronized charcoal. The micronized charcoal used has a particle size of 2.5 microns (PM 2.5) or less that mimics the small particulates found in polluted air. Each treatment area was washed five times using deionized water. Images were taken pre- and post-wash using a dissecting microscope.

The test material consisted of 2% **ACB Yerba Santa Glycoprotein PF** in a Cetaphil Moisturizing for All Skin Types. For added perspective, images of an untreated test site and a site treated with Cetaphil Moisturizing for All Skin Types were recorded.

Color analysis was conducted on the images and results depicted in optical density values and pigmentation histograms. Images were inverted and standard coloration values recorded and assigned absorbance units. The lower the mean optical density value the better protection against carbon particle embedment or PM 2.5 inhibition.

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Results

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

ACB Yerba Santa Glycoprotein PF at a concentration of 2% was able to provide protection from carbon pollution.

Figure 1: ACB Yerba Santa Glycoprotein PF Histogram Images - Inhibition on PM 2.5

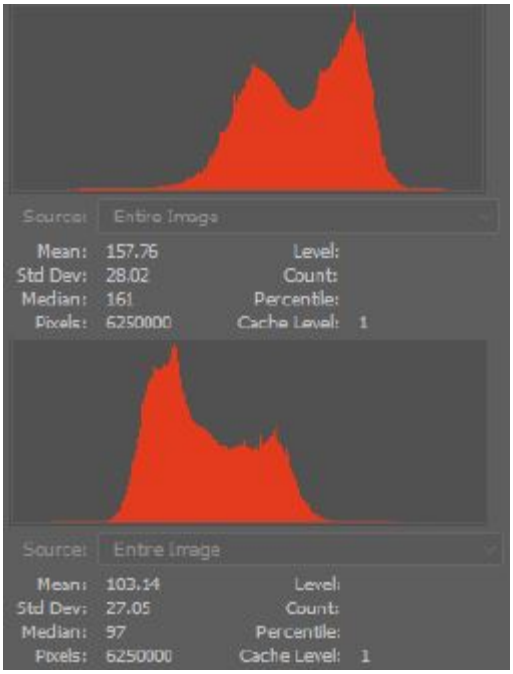

Figure 2: **ACB Yerba Santa Glycoprotein PF** Images

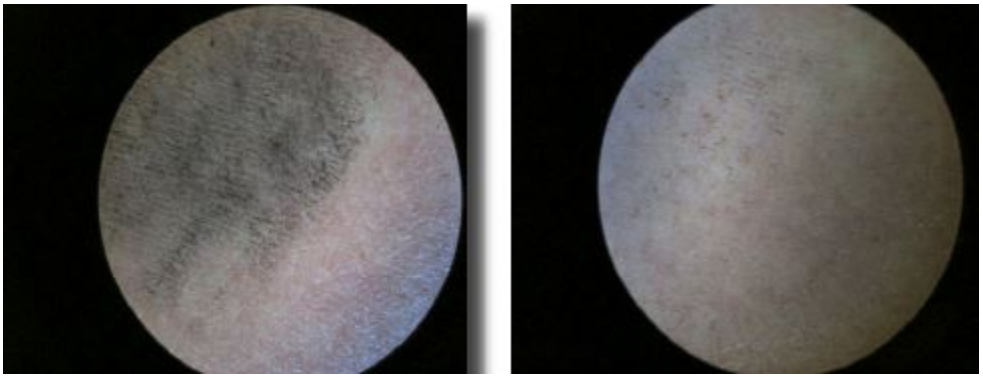

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Figure 3: Untreated Histogram Images - Inhibition on PM 2.5

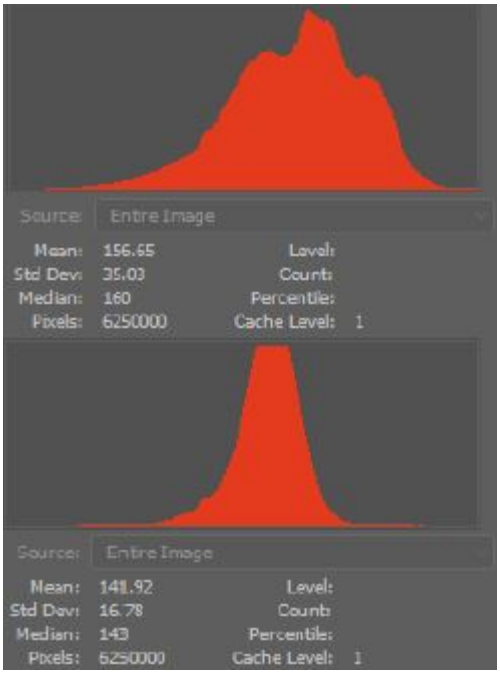
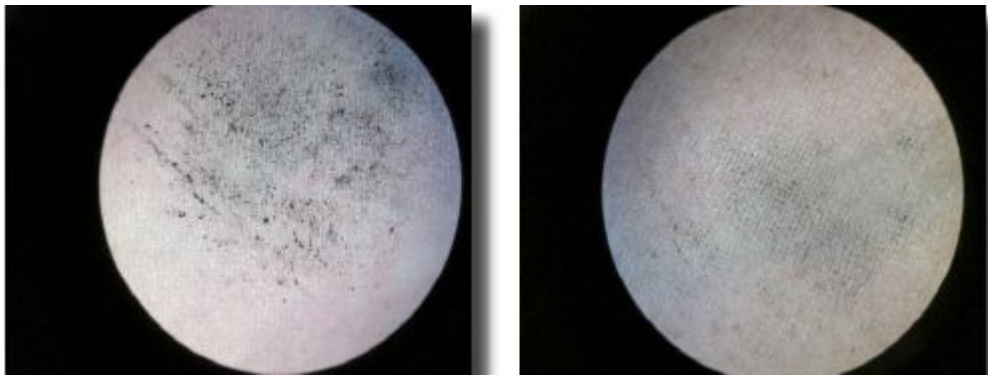


Figure 4: Untreated Images



Discussion

As shown in figure 1, **ACB Yerba Santa Glycoprotein PF (code 20342PF)** was able to provide pollution protection as specified by micronized carbon residue. The small amount of carbon that remains compared to the untreated control indicates the ability of **ACB Yerba Santa Glycoprotein PF** to provide barrier protection against everyday air pollution and slow the extrinsic aging process. It can therefore be concluded that at normal use concentrations **ACB Yerba Santa Glycoprotein PF** can be used as a skin pollution protection active ingredient.