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ACB Wasabi Extract PF
Efficacy Data

Code: 20351PF
INCI Name: Lactobacillus/Wasabia Japonica Root Ferment Extract
CAS #: N/A
EINECS #: N/A

Type of Study	Results
ORAC Assay	Oxygen Radical Absorbance Capacity (ORAC) is a measure of a materials potential to protect against oxidative stress or reactive oxygen species (ROS). **ACB Wasabi Extract PF** demonstrated significant antioxidant activity by reducing the presence of ROS compared with Trolox, the vitamin E analog.
SOD Test	A Xanthine Oxidase based model was used to evaluate the pseudo enzymatic antioxidant activity of **ACB Wasabi Extract PF.**
Anti-microbial Challenge Test	An anti-microbial challenge test was conducted to evaluate **ACB Wasabi Extract PF's** anti-microbial activity against broad-spectrum microorganisms.

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Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: ACB Wasabi Extract

Code: 20351PF

CAS #: N/A

Test Request Form #: 62

Lot #: 26056

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 Wasabi 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 **ACB Wasabi 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

ACB Wasabi Extract began exhibiting antioxidant activity at 0.015%.

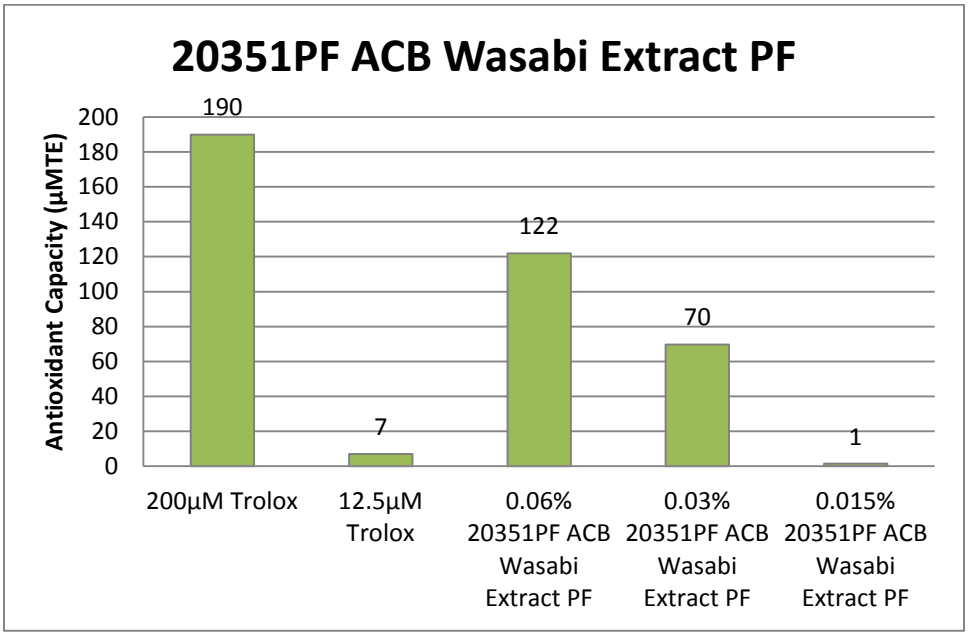

Figure 1: Antioxidant capacities

Discussion

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

ACB Wasabi Extract was designed to provide anti-microbial and anti-oxidant properties. With the present study we can confirm that this unique ingredient is capable of providing functional and potent antioxidant benefits when added to cosmetic applications.

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ACB Wasabi Extract PF
SOD-Active Measurement Test

Code: 20351PF
INCI Name: Lactobacillus/Wasabia Japonica Root Ferment Extract
Suggested Use Levels: 1.0 - 5.0%

Abstract

A Xanthine Oxidase based model was used to evaluate the pseudo enzymatic antioxidant activity of **ACB Wasabi Extract PF.**

Materials and Methods

NBT method (Nitro Blue Tetrazolium)
Samples
ACB Wasabi Extract PF
1% (L)-(+)-Ascorbic Acid Solution

Reagents:

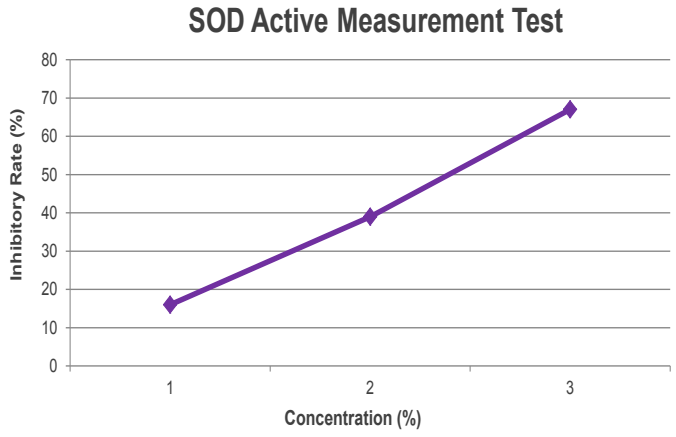
1) 0.05M Na2Co3 buffer (pH10.2)	2.4ml
2) 3mM Xanthine	0.1ml
3) 3mM EDTA	0.1ml
4) 0.1% BSA(Bovine Serum Albumin)	2.0ml
5) 0.75mM NBT(Nitro Blue Tetrazolium)	0.1ml
6) Xanthine Oxidase (from Bovine Milk)	0.1ml
7) 6mM CuCl2	0.1ml

Procedure:
1) Added 1 & 5 Reagents & sample 0.1ml to test tube
2) Stabilized for 10min at 25 C
3) Added Xanthine Oxidase & incubated for 20min at 25 C
4) Added 7
5) Meausured absorbance of 560nm (At)
6) Measured absorbance of blank (AB) & Control (Ao)
7) Calculated inhibitory rate (%)

$$\text{Inhibitory Rate (\%)} = \frac{\text{Ab-(At-Ao)}}{\text{Ab}} \times 100$$

*Blank: water was used for sample
*Control: water was used for Xanthine oxidase

Results



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Inhibition Activity Data

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Product Name: ACB Wasabi Extract PF / ACB Wasabi Extract SF
Code Number: 20351PF / 20015EIK
Lot Number: 32177P / NC131016-A
CAS #'s: N/A
EINECS #'s: N/A
INCI Name: *Lactobacillus/Wasabia japonica* Root Ferment Extract

Organism (ATCC #)	Minimum Inhibitory Concentration (%)	
	ACB Wasabi Extract PF	ACB Wasabi Extract SF
E.coli #8379	4.0	4.0
S. aureus #6538	4.0	4.0
P. aeruginosa #9027	8.0	8.0
C. albicans #10231	2.0	2.0
A. brasiliensis #16404	2.0	2.0

QA Signature Monica Beltran

Date 12/12/2013

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