ACB Lemon Peel Extract G
Efficacy Data

Code: 20364G
INCI Name: Glycerin & Water & Lactobacillus/ Lemon Peel Ferment Extract
CAS #: 56-81-5 & 7732-18-5 & 84929-31-7
EINECS #: 200-289-5 & 231-791-2 & 284-515-8

Name of Study	Results
Tyrosinase Inhibition Assay	Based on the results of the tyrosinase inhibition assay conducted using 2.0% ACB Lemon Peel Extract G, the product is capable of inhibiting tyrosinase 34.0% better than hydroquinone when measured with a Shimadzu UV-1601 UV/Vis Spectrophotometer.

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Tyrosinase Inhibition Assay

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Tradename: ACB Lemon Peel Extract G

Code: 20364G

CAS #: 56-81-5 & 7732-18-5 & 84929-31-7

Lot #: NC140710-D

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

Test Performed:
Tyrosinase Inhibition Assay

Introduction

Tyrosinase is a key enzyme in melanin biosynthesis, involved in determining the color of mammalian skin and hair. Tyrosinase's main application is to identify new potent tyrosinase inhibitors in the cosmetic industry. Tyrosinase is a copper-containing monooxygenase that is widely distributed in nature. The enzyme catalyzes the first two reactions of melanin synthesis, the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine, L-dopa, and the oxidation of L-dopa to dopaquinone. This quinone is a highly reactive compound and can polymerize spontaneously to form melanin. Tyrosinase is one of the causes of hyperpigmentation, an over-production of dermal melanin pigment, leading to melasmas, freckles, age-spots, and liver spots.

A tyrosinase inhibition assay was conducted on **ACB Lemon Peel Extract G** to assess its ability to inhibit tyrosinase, thus indicating its use to reduce hyperpigmentation.

Assay Principle

This assay is based on the conversion of L-tyrosine into a dopachrome complex by tyrosinase. This dopachrome complex has an absorbance at 490nm and can be quantitated through optical density measurements. The greater the inhibition exhibited by the sample, the lower the optical density value due to the lack of L-tyrosine conversion.

$$\text{L-Tyrosine} + O_2 \text{ ---(Tyrosinase)-}\rightarrow \text{L-DOPA}$$

$$\text{L-DOPA ---(Tyrosinase)-}\rightarrow \text{L-DOPA-quinone} + H_2O$$

L-DOPA = L-3,4-Dihydroxyphenylalanine

Active Concepts

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Tyrosinase Inhibition Assay

Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	Phosphate Buffered Saline, PBS (0.1M)
C. Reagents:	Mushroom Tyrosinase (1000U/mL); L-Tyrosine (1mM); Hydroquinone USP; **ACB Lemon Peel Extract G**; Licorice Extract/Polysorbate-80 (1:4)
D. Preparation:	Pre-heat (37°C) Synergy H1 Microplate reader
E. Plates:	96 Well Microtitre Plates

Methods

Solutions of **ACB Lemon Peel Extract G** (0.05%, 0.10%, 0.50%, 2.00%), licorice extract, hydroquinone, L-tyrosine, and mushroom tyrosinase were prepared in 0.1M Phosphate Buffered Saline. Phosphate Buffered Saline was used as the negative control. For the inhibition assay, 10µL of test material and controls were combined with 170µL of 1mM L-tyrosine and 20µL 1000U/mL mushroom tyrosinase in a 96- well microtitre plate. The plate was placed in the Synergy H1 reader set to 37°C and optical density measurements were then taken every minute for 20 minutes at 490nm.

The percent of tyrosinase inhibition was calculated by the below equation:

$$\%\ Inhibition = \frac{Optical\ Density_{PBS} - Optical\ Density_{Sample}}{Optical\ Density_{PBS}} \times 100$$

Results

	0.05% Concentration	0.1% Concentration	0.5% Concentration	2.0% Concentration
ACB Lemon Peel Extract G	8	33	56	71
Hydroquinone	2	11	27	53
Licorice Extract	6	28	52	68
ACB Lemon Peel Extract G vs. Hydroquinone	300	200	107.4	34
ACB Lemon Peel Extract G vs. Licorice Extract	33	18	8	4

Table 1. Percent (%) Tyrosinase Inhibition.

ACB Lemon Peel Extract G was able to inhibit tyrosinase 34.0% better than hydroquinone at the highest concentration tested.

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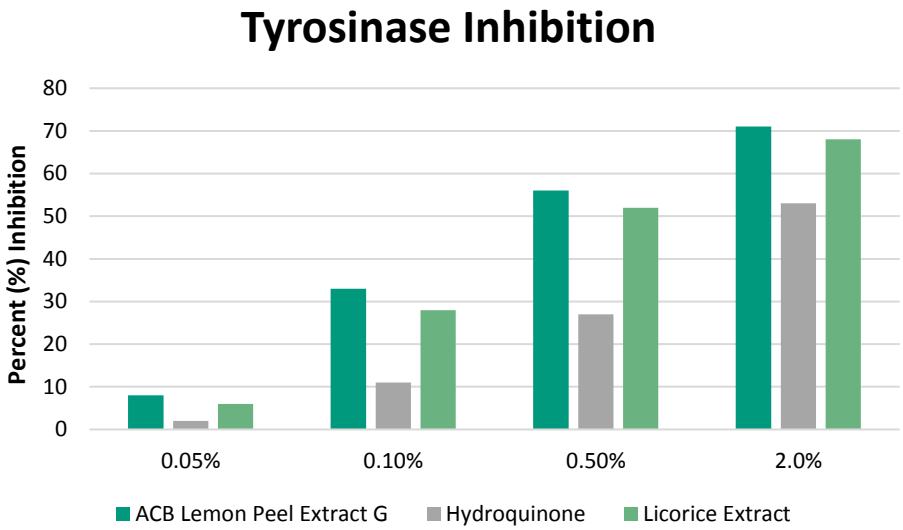

Graph 1. Percent (%) Tyrosinase Inhibition.

Discussion

As shown in Graph 1, **ACB Lemon Peel Extract G** was able to inhibit tyrosinase compared to hydroquinone and licorice extract. The inhibition activity of **ACB Lemon Peel Extract G** increased as the concentration increased, as a result we can assure that its ability to decrease tyrosinase activity is dose dependent. With the present study we can confirm that this unique ingredient is capable of providing skin lightening benefits to counteract problems associated with hyperpigmentation.