Tyrosinase Inhibition Assay

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Tradename: AC ExoEternol

Code: 60200

CAS #: 7732-18-5 & 68333-16-4 (or) 92128-79-5 & 90082-61-4 (or) 68132-21-8 & 123465-35-0 (or) 8002-43-5 & 68333-16-4 (or) 1686112-36-6 (or) 9015-54-7

Test Request Form #: 13038

Lot #: N250701O

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

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 to determine the ability of **AC ExoEternol** to inhibit tyrosinase, indicating its potential in cosmetic applications 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 510nm 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. This is a time course assay, after which the results are analyzed and compared to a known tyrosinase inhibitor, Kojic Acid.

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Materials

- **A. Kit:** Tyrosinase Inhibition Screening Kit, Colorimetric (Sigma; MAK257)*
- **B. Equipment:** Synergy H1 Microplate reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instruments, Winooski, VT); Pipettes
- **C. Buffers:** Tyrosinase Assay Buffer (MAK257A)*
- **D. Reagents:** Tyrosinase Substrate (MAK257B)*; Tyrosinase (MAK257C)*; Tyrosinase Enhancer (MAK257D)*; Inhibitor Control Kojic Acid (0.01% (0.75mM), MAK257E)*
- **E. Software:** Excel Analysis ToolPak (Microsoft)
- **F. Preparation:** Synergy H1 Microplate reader
- **G. Plates:** 96 Well Microtiter Plates; Multichannel sample wells

*Or suitable alternatives, subject to change without notice based off vendor availability

Methods

Solutions of **AC ExoEternol** (1.25%, 2.5%, 5.0%), 0.01% Kojic Acid (positive inhibitor control), tyrosinase substrate solution, and tyrosinase enzyme solution were prepared in tyrosinase assay buffer. For the negative control, tyrosinase assay buffer was used and is labeled as the Enzyme Activity Control. 20 µL of test material and controls were combined with 50 µL of the tyrosinase enzyme solution and incubated at 25°C for 10 minutes. Next, 30 µL of the tyrosinase substrate solution was added to each respective test well. The plate was placed in the Synergy H1 reader and optical density measurements were then taken every minute for 60 minutes at 510 nm.

The slope of each sample was calculated by the following equation:

$$Slope = \frac{\Delta\text{Absorbance } (\text{Abs}_2 - \text{Abs}_1)}{\Delta\text{Time } (\text{T}_2 - \text{T}_1)} \times 100$$

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

$$\%\ Inhibition = \frac{Slope_{EC} - Slope_{Sample}}{Slope_{EC}} \times 100$$

Assays were repeated three separate times with each sample run in duplicate. Duplicates for each replicate were averaged, and the average of all three experiments is displayed. Data was analyzed using a one-way ANOVA with statistical significance accepted at $p \leq 0.05$.

Results

The data obtained from this study met criteria for a valid assay and the control performed as anticipated. All concentrations of **AC ExoEternol** inhibited tyrosinase in a dose dependent fashion.

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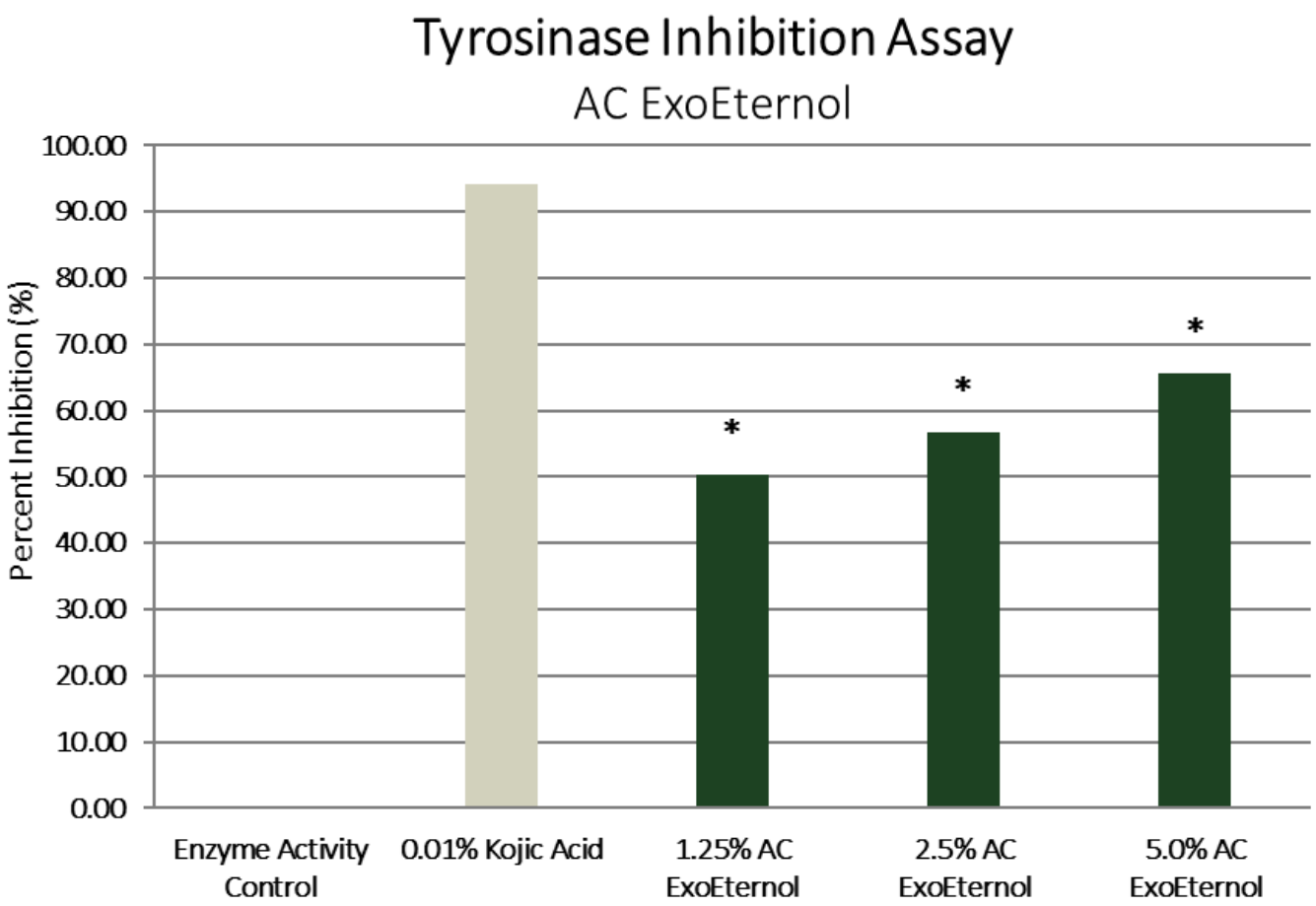

Figure 1. Tyrosinase Inhibition. * indicates significance ($p \leq 0.05$) compared to Kojic Acid.

Table 1. Results from one-way ANOVA Statistical Analysis Compared to 0.01% Kojic Acid. * indicates significance ($p \leq 0.05$) compared to Kojic Acid.

	Enzyme Activity Control	1.25% **AC ExoEternol**	2.5% **AC ExoEternol**	5.0% **AC ExoEternol**
P-value	< 0.001*	0.001*	< 0.001*	0.001*

Discussion

As shown in Figure 1 and Table 1, Kojic Acid, significantly inhibited tyrosinase by 94%. This data demonstrates the cosmetic industry standard skin lightening active ingredient (Kojic Acid) provides skin lightening benefits by inhibiting tyrosinase.

Similarly, **AC ExoEternol** at 1.25%, 2.5%, and 5.0% inhibited tyrosinase by 50%, 57%, and 66%, respectively. The inhibition activity of **AC ExoEternol** increased as the concentration increased, confirming the ability to decrease tyrosinase activity is dose dependent (Figure 1).

Collectively, these data indicate **AC ExoEternol** inhibits tyrosinase activity and can provide skin lightening benefits to counteract challenges associated with hyperpigmentation.