

ACTIVE CONCEPTS LLC

107 Technology Drive, Lincolnton | NC 28092 USA • <u>info@activeconceptsllc.com</u> • Phone: +1-704-276-7100 • Fax: +1-704-276-7101 • <u>https://activeconceptsllc.com/</u>

Tradename: ABS Willow Bark Extract

<u>Code:</u> 10200

CAS #: 7732-18-5 & 84650-64-6

Test Request Form #: 10649

Lot #: 9392455

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092 **Study Director:** Maureen Drumwright **Principal Investigator:** Kayla Patterson

Test Performed:

Cellular Renewal Study

Introduction

As the largest human organ, the skin's integrity is critical to properly function as a physical barrier and maintenance of a heathy appearance for aesthetics. The epidermis constantly undergoes major self-renewal as the superlayer of cells are lost by desquamation and replaced by cells in the basal layers. The constant replacement of cells mitigates the negative long-term effects of ultraviolet light damage and harmful agents (chemicals, pollutants, etc.) on the skin. Aiding in the processes of cellular renewal can improve the skin's physical appearance as well as function as a protective barrier.

Accordingly, a cellular renewal study was conducted to evaluate the ability of **ABS Willow Bark Extract** to accelerate skin cell replacement by assessing changes in pigmentation.

Study Principle

Dermal Dye Max[™] (active ingredient: dihydroxyacetone (DHA) / glycerone) is applied to the skin and creates artificially high pigmented skin. The controls and test materials are applied to the artificially pigmented areas and pigmentation is measured overtime. The artificially pigmented areas provide a model to assess cellular renewal by measuring pigmentation with decreases in pigmentation representing cellular renewal.

Materials

- A. Equipment: DermaLab Skin Combo (Skin Color Probe); Digital Camera
- **B.** Products: Dermal Dye Max[™] (Alpine Valley Naturals); Base Lotion (Cetaphil[®] Moisturizing Cream for All Skin Types); Glycolic Acid (positive control)



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Methods

Volunteers between the ages of 22 and 43, who were known to be free of any skin pathologies with Fitzpatrick skin types I to III, participated in this study (Table 1).

Fitzpatrick Skin Type Descriptions*				
Skin Type	Description			
I	Always burns, never tans			
II	Burns easily, tans minimally			
III	Burns moderately, tans to light brown			
IV	Burns minimally, tans to moderate brown			
V	Rarely burns, tans to dark			
VI	Never burns, least sensitive to changes			
*Adapted from The Surgeon General's Call to Action to Prevent Skin Cancer				

 Table 1. The Fitzpatrick Classification of Skin Types Chart¹

Five test sites were identified on the volar forearm of participants. The first test site was identified as the Comparative Skin Site, and no dye nor treatment were applied to this site to demonstrate normal pigmentation readings. Dermal Dye Max^{TM} was applied to the remaining four test sites and allowed to develop for 24 hours prior to baseline readings. After dye development and prior to initial treatment application, baseline DermaLab pigmentation index readings were taken for all five identified sites. The skin test site conditions and treatments are described below (Table 2). The Untreated Dye Control received Dermal Dye Max^{TM} but no treatment to demonstrate normal cellular renewal.

All lotion formulations and the Base Lotion were adjusted to a direct pH of 5.0 - 5.5 (Table 2). The Base Lotion utilized in this study was Cetaphil[®] Moisturizing Cream for All Skin Types. Approximately 0.2 g of each treatment was applied to three 2 cm x 2 cm respective locations on the volar forearm. Pigmentation readings and images were taken every 24 hours until the active test site returned to baseline. After each daily reading, treatment of each respective test site was performed following the same parameters listed above.

Skin Test Site	Condition	Dermal Dye Max [™] Application?	Treatment / Test Material Application Description	Lotion pH
1	Comparative Skin Site	No	None	N/A
2	Untreated Dye Control	Yes	None	N/A
3	Base Lotion	Yes	Base Lotion	5.3
4	2.0% Glycolic Acid	Yes	2.0% Glycolic Acid in Base Lotion	5.2
5	2.0% ABS Willow Bark Extract	Yes	2.0% ABS Willow Bark Extract in Base Lotion	5.3

The percent change of Pigmentation values was calculated for each test site at every timepoint relative to Baseline values, using the following equation:

 $Percent Change (\%) = \frac{Pigmentation Index_{Day} - Pigmentation Index_{Baseline}}{Pigmentation Index_{Baseline}} \times 100$



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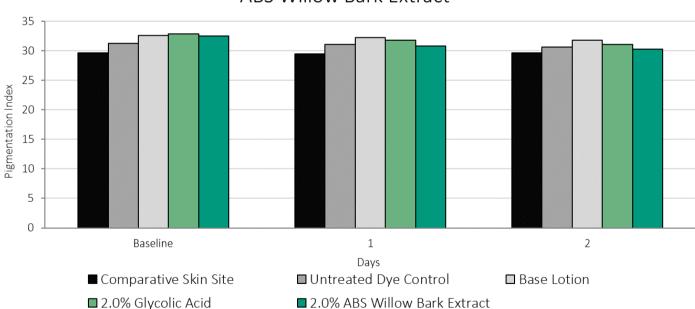
Cumulative Cellular Renewal, represented by the Pigmentation Index values and relative to the Untreated Dye Control, was calculated as area under the curve (AUC) by using the following equation:

$$AUC = \frac{1}{2} \sum_{i=0}^{n-1} (t_{i+1} - t_i) (Pigmentation \, Index_i + Pigmentation \, Index_{i+1})$$

For pigmentation measurements *Pigmentation Index1* and *Pigmentation Index2* at times *t1* and *t2*, the AUC between those two time points is equivalent to the product of difference in time and the average of the two Pigmentation measurements. Provided Pigmentation Index values decreased over time AUC was calculated as an inverse and presented as a positive value to demonstrate the amount of cellular relative to the Untreated Dye Control.

<u>Results</u>

The data obtained from this study met criteria for a valid study as the Comparative Skin Site, Untreated Dye Control, and Glycolic Acid performed as anticipated. Application of 2.0% **ABS Willow Bark Extract** accelerated cellular renewal as pigmentation values returned to baseline levels after two days.



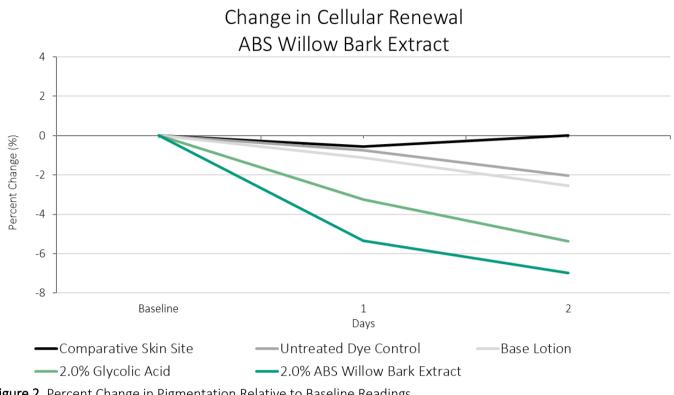
Cellular Renewal ABS Willow Bark Extract

Figure 1. Cellular Renewal of Pigmentation Index Values Overtime



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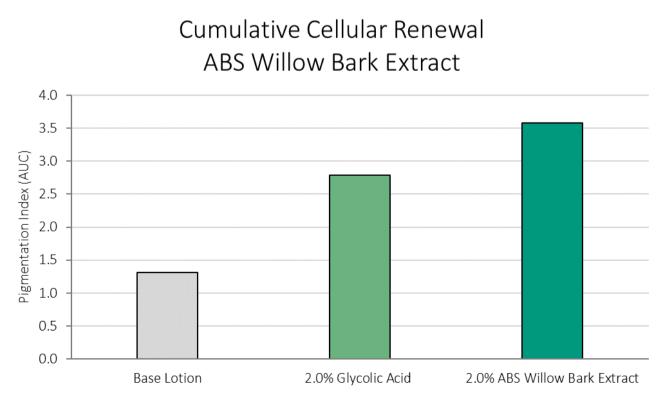
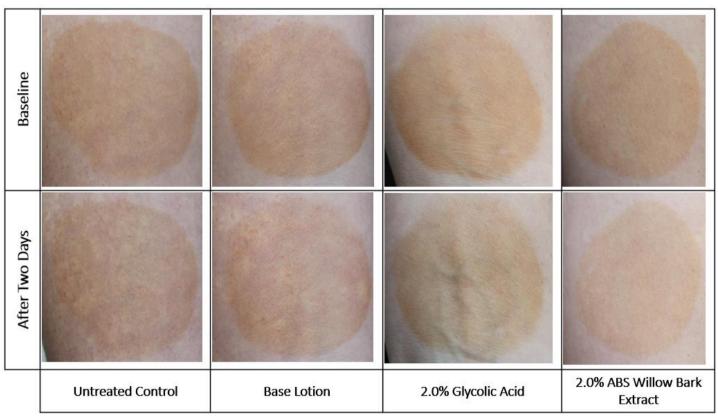


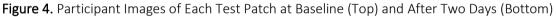
Figure 3. Cumulative Cellular Renewal in Pigmentation Relative to the Untreated Dye Control



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Discussion

The ability of **ABS Willow Bark Extract** to accelerate skin cellular renewal was assessed through changes in pigmentation. As shown in Figure 1 and 2, the Dermal Dye Max[™] artificially augmented pigmentation values at baseline measurements. After two days, the Untreated Dye Control demonstrated a 2% reduction in pigmentation indicating normal cellular renewal (Figures 1, 2). Similarly, the Base Lotion exhibited a 3% decrease in pigmentation demonstrating a slight increase in normal cellular renewal. After two days of application, 2.0% Glycolic Acid induced a 5% decrease in pigmentation as expected. However, 2.0% **ABS Willow Bark Extract** elicited a 7% reduction in pigmentation and outperformed the positive control.

Similar results are shown when the data is displayed to illustrate the collective effect of each treatment cellular renewal when the Untreated Dye Control pigmentation values are accounted for. As shown in Figure 3, 2.0% ABS Willow Bark Extract produced the largest change in cumulative cellular renewal compared to the Base Lotion and % Glycolic Acid. Examining images of the skin test sites at Baseline and after two days of the application, 2.0% ABS Willow Bark Extract visually outperformed the Base Lotion and 2.0% Glycolic Acid in terms of reducing pigmentation.

Taken together, these results indicate **ABS Willow Bark Extract** enhances cellular renewal when added to personal care applications at recommended use levels. Collectively, **ABS Willow Bark Extract** accelerates the processes of cellular renewal which can improve the skin's physical appearance as well as function as a protective barrier.

References

1. Sharma AN, Patel BC. Laser Fitzpatrick Skin Type Recommendations. [Updated 2022 Mar 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557626/