

## Targeting Cellular Senescence: A Novel Strategy for Multi-Age Skin Health Improvement Using *Casearia sylvestris* and *Hymenaea courbaril* Extracts

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### Abstract

The skin undergoes significant physiological changes with aging, resulting in chronic inflammation, compromised barrier function, and impaired production of collagen and elastic fibers. A key driver of these changes is the accumulation of senescent cells, which exhibit reduced proliferation, resistance to apoptosis, and secretion of inflammatory factors collectively known as the senescence-associated secretory phenotype (SASP). These factors contribute to tissue deterioration and visible skin aging. Targeting senescent cells has emerged as a promising strategy to counteract aging and improve skin health. This study investigates the efficacy of two bioactives, *Casearia sylvestris* and *Hymenaea courbaril* extract, in addressing age-related skin changes and promoting multi-age efficacy by targeting senescence pathways. Previous studies from our research group showed the ability of both bioactives to act on cellular senescence pathways, and in stimulate elastin and collagen production, using primary human dermal fibroblasts irradiated with UVB radiation. To explore their multi-age efficacy, a clinical trial was conducted on 302 Brazilian women (aged 30-79 years, Fitzpatrick phototypes II–VI), divided into four age groups (30-44, 45-59, 60-69, and 70-79 years). Subjects applied a cosmetic formulation containing these bioactives once daily for 28 days. Skin parameters assessed included hydration, pH, barrier function, wrinkle depth, *in vivo* stimulation of collagen and elastin, and skin responsiveness. After 28 days, significant improvements were observed across all age groups. Skin hydration and barrier function were enhanced, while pH levels remained constant. Wrinkle analysis revealed a visible reduction, and *in vivo* collagen and elastin synthesis increased significantly after treatment. This is the first study to demonstrate the clinical potential of senescence-targeting bioactives in a diverse, multi-life stage population. By translating their biological mechanisms into effective cosmetic formulations, this research highlights the promise of a novel approach to combat age-related skin changes and inflammation. The findings pave the way for the development of targeted solutions to prevent cellular senescence and promote skin health, providing significant benefits to diverse populations.

### 1. Introduction

Skin aging is a multifactorial biological process involving both intrinsic and extrinsic mechanisms. Intrinsic aging, also referred to as chronological aging, is driven by genetic and metabolic factors and characterized by thinning of the dermis, loss of structural proteins, and decreased cellular turnover. Extrinsic aging, primarily induced by ultraviolet (UV) radiation, pollution, and lifestyle factors, accelerates these changes through oxidative stress and inflammation [1,2].

A growing body of evidence highlights the accumulation of senescent cells as a key contributor to both intrinsic and extrinsic skin aging. Cellular senescence is a stable cell-cycle arrest state that cells enter in response to various stressors, including DNA damage, oxidative stress, and telomere shortening [3]. Although initially protective, the persistent presence of senescent cells in tissues is deleterious due to the secretion of a myriad of inflammatory mediators, growth factors, and matrix-degrading enzymes collectively termed the senescence-associated secretory phenotype (SASP) [4].

Traditional approaches to mitigating skin aging have focused on antioxidation, hydration, and stimulation of collagen synthesis. However, emerging strategies now seek to address the upstream drivers of aging, notably by targeting cellular senescence. Therapeutic interventions can be broadly categorized into “senolytics,” which selectively eliminate senescent cells, and “senomorphics,” which suppress the deleterious SASP while preserving cell viability [5]. Botanical compounds offer a particularly promising avenue for senescence modulation, given their complex phytochemical compositions and long-standing use in traditional medicine.

In this study, we investigate the efficacy of two botanical extracts: *Casearia sylvestris* and *Hymenaea courbaril*, as senescence-targeting agents for skin health improvement. Our previous *in vitro* research demonstrated that these extracts could reduce markers of senescence and stimulate extracellular matrix protein production in UVB-irradiated dermal fibroblasts.

The goal of this research is to evaluate whether a formulation containing these bioactives can offer multi-age, multi-ethnic benefits in mitigating signs of aging through modulation of cellular senescence pathways. This work also seeks to expand the paradigm of anti-aging skincare beyond symptomatic treatment toward addressing the biological roots of aging.

## 2. Materials and Methods

### 2.1. Ethical compliance

The clinical trials were conducted under Resolution 466/12 of the National Council of Health on Regulatory Guidelines and Standards for Research Involving Humans. Dermatological and ophthalmological monitoring was maintained throughout the protocol. The research protocol received approval from the Institutional Ethics Committee, with second opinion numbers 5.580.085, 5.580.056, 5.579.913, 5.579.854, 5.754.508, 5.039.131, 5.675.912, and 5.678.846. All subjects in this research had given their informed consent to participate.

### 2.2. Test Materials

A total of 302 healthy female volunteers aged 30 to 79 years with Fitzpatrick skin phototypes II to VI were enrolled. Participants were stratified into four age groups: 30–44 years, 45–59 years, 60–69 years, 70–79 years. A cosmetic formulation containing *Casearia sylvestris* and *Hymenaea courbaril* extracts was developed, and subjects applied the product once daily, for over 28 days.

### 2.3. Skin hydration

Long-term skin hydration was evaluated by randomly applying the product to the face. Measurements were obtained using a Corneometer (Courage+Khazaka Electronic GmbH, Germany) at D0 and D28.

### 2.4. Transepidermal water loss evaluation

Transepidermal water loss (TEWL) was measured using the evaporimetry technique with a Tewameter (Courage+Khazaka Electronic GmbH, Germany) on the forearm. Measurements were taken at D0 and D28.

### 2.5. Skin pH

Skin pH was performed on the forearms at D0 and D28, and it was assessed with a Skin-pHmeter® probe coupled to a Multi Probe Adapter (Courage+Khazaka Electronic GmbH, Germany).

### 2.6. Wrinkle evaluation

Wrinkle reduction was assessed using high-resolution digital photographs taken under controlled conditions at D0 and D28. Images were captured with both smiling and relaxed facial expressions. The intensity of wrinkles (R) was determined by analyzing a

specified area at the outer corner of each subject's eye. Higher R values indicated greater wrinkle intensity.

### 2.7. Collagen and elastin synthesis

The product was randomly applied in the forearm, once a day, for 28 d. The control group was set as the skin without application of any product. Anti-aging benefits were evaluated by the protein levels of type-I pro-collagen and elastin.

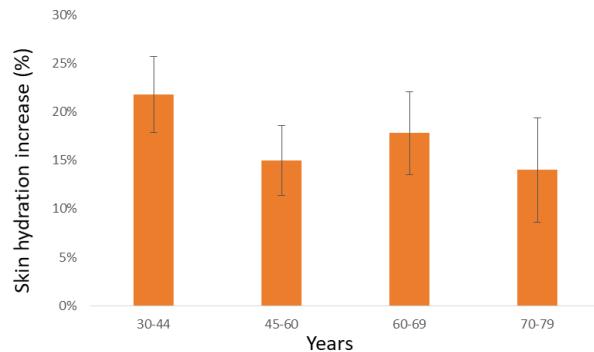
### 2.8. Statistical Analysis

The significance of changes in skin biophysical parameters was assessed using the paired Student's t-test with a 95% confidence interval. Statistical analyses were conducted using GraphPad Prism 8.00 software (GraphPad Software, USA). Values obtained at D0 were compared to those obtained at D28.

## 3. Results

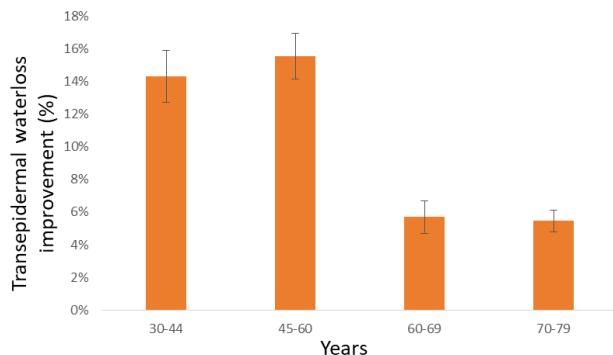
### 3.1. Improvements in Skin Hydration and Barrier Function

After 28 days of treatment, all age groups showed a significant improvement in skin hydration. Corneometry measurements (Figure 1) increased by 21.8% in the 30–44 age group, 15% in the 45–60 group, 17.8% in the 60–69 group, and 14% in the 70–79 group.



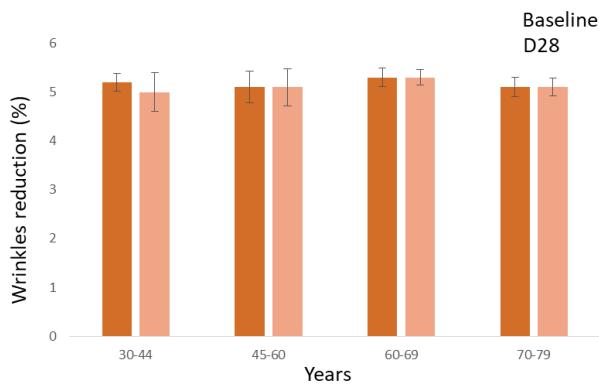
**Figure 1.** Increased percentage of skin hydration at D28. Bar graphs represent the mean values of 12, 22, 22, and 11 subjects for group ages 30–44, 45–60, 60–69, and 70–79 respectively. Data were analyzed using bimodal paired Student's t-test ( $p<0.001$ ).

Improvements in barrier function (Figure 2) were also observed, with increases of 14.3%, 15.6%, 5.7%, and 5.5% in the respective age groups.



**Figure 2.** Increased percentage of barrier function at D28. Bar graphs represent the mean values of 44, 51, 46, and 46 subjects for group ages 30–44, 45–60, 60–69, and 70–79 respectively. Data were analyzed using bimodal paired Student's t-test ( $p<0.001$ ).

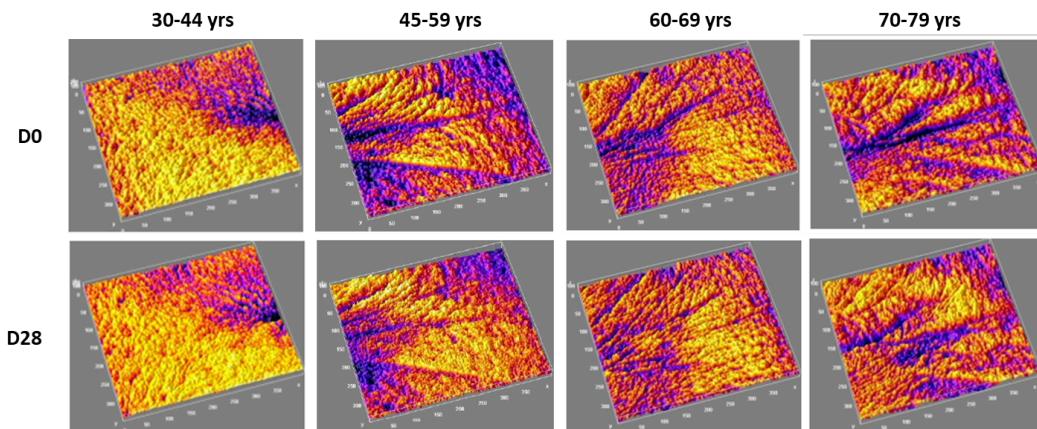
Skin pH levels remained stable throughout the study, highlighting the formulation's mild and skin-friendly nature.



**Figure 3.** Mean values of skin pH. The measurements were obtained on the forearms at baseline (D0) and after 28 days of product use (D28). Bar graphs represent the mean values of 12, 10, 21, and 16 subjects for group ages 30-44, 45-60, 60-69, and 70-79 respectively. Data were analyzed using bimodal paired Student's t-test. No statistical differences were found among the groups.

### 3.2. Reduction of Wrinkle Depth

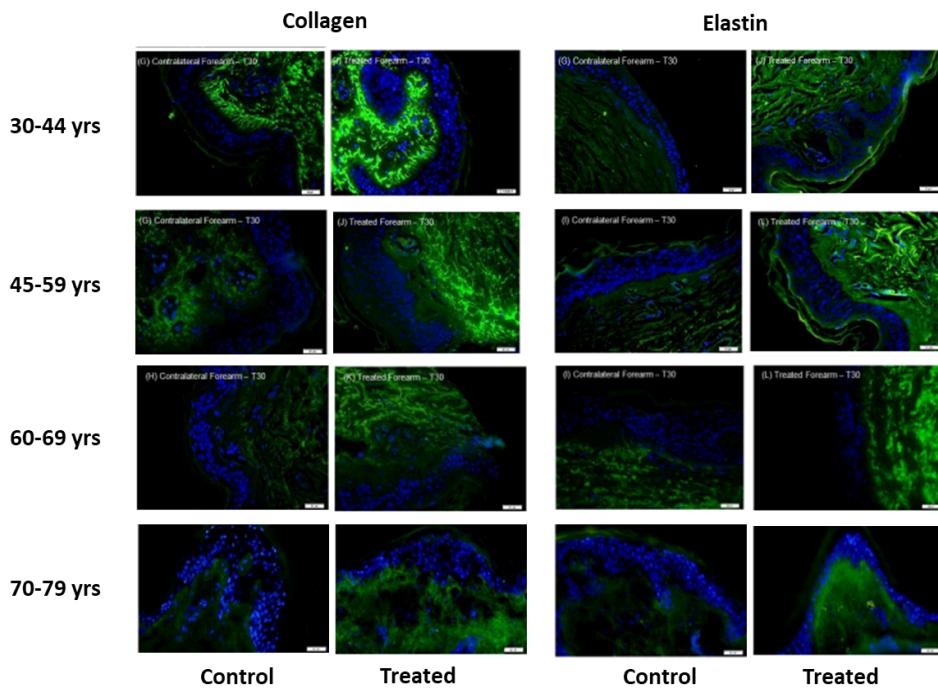
There was a significant reduction in wrinkle intensity after 28 days of product use, with reduction of 6.2%, 6.2%, 7.7%, and 6.7% in the respective age groups (Figure 4).



**Figure 4.** Wrinkle reduction assessed by image analysis. Representative image obtained from subjects before product use (D0) and after 28 days of product treatment (D28).

### 3.3. Enhancement of Collagen and Elastin Production

After 28 days of product use, we found that both biomarkers evaluated were significantly increased, as follows: type I pro-collagen (9.8%, 82.0%, 101.9%, and 88.4% in the respective age group); and elastin (7.1%, 86.2%, 52.9%, and 61.7% in the respective age group) (Figure 5).



**Figure 5.** Immunofluorescence evaluation of biological markers in skin biopsies 28 d after treatment. Molecules of interest are stained in green and blue color corresponding to the cell nucleus. The reference bar corresponds to 10  $\mu$ m.

#### 4. Discussion

This study provides robust clinical evidence supporting the concept that targeting senescent cells can yield visible and measurable improvements in skin aging parameters. The observed effects on hydration, barrier reinforcement, wrinkle reduction, and ECM biosynthesis are congruent with the biological processes modulated by senescence and SASP factors.

Importantly, the broad efficacy across different age groups and skin phototypes reinforces the universal relevance of cellular senescence in skin aging. By maintaining skin pH and ensuring excellent tolerability, the formulation achieved meaningful biological activity without compromising the skin's delicate homeostasis.

These findings align with a growing scientific consensus that interventions targeting fundamental aging processes — rather than merely symptoms — offer the most promising path toward healthier, more resilient skin [6].

#### 5. Conclusion

Targeting cellular senescence represents an innovative and effective strategy for improving skin health and mitigating the signs of aging. The present study demonstrates that a formulation containing Casearia sylvestris and Hymenaea courbaril extracts delivers significant benefits in hydration, barrier function, wrinkle reduction, and extracellular matrix regeneration across a wide demographic. These findings highlight the potential of senescence-modulating bioactives to usher in a new era of science-driven cosmetic innovation focused on the fundamental biology of aging.

#### References

1. Campisi, J. (2013). Aging, Cellular Senescence, and Cancer. *Annual Review of Physiology*, 75, 685–705.
2. Krutmann, J., et al. (2017). The skin aging exposome. *Journal of Dermatological Science*, 85(3), 152–161.

3. López-Otín, C., et al. (2013). The Hallmarks of Aging. *Cell*, 153(6), 1194–1217.
4. Coppé, J.P., Desprez, P.Y., Krtolica, A., Campisi, J. (2010). The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annual Review of Pathology*, 5, 99–118.
5. Kirkland, J.L., Tchkonia, T. (2017). Cellular Senescence: A Translational Perspective. *EBioMedicine*, 21, 21–28.
6. Birch, J., Gil, J. (2020). Senescence and the SASP: many therapeutic avenues. *Genes & Development*, 34(23-24), 1565–1576.