IFSCC 2025 full paper (IFSCC2025-1313)

Enhancing Skin Longevity through Stem Cell Protection and Senescence Modulation: A Botanical Approach for Anti-Aging and Regeneration

Stéphanie Brédif [1,*], Lucille Blanchot [1], Chloé Bordron [1], Sophie Zozine [1], Virginie Pecher [1] and Virginie Fera [1]

1 Groupe Rocher, Scientific Direction, Issy-les-Moulineaux, France

1. Introduction

Skin aging is a complex process influenced by intrinsic factors, such as genetic predisposition, and extrinsic factors, including UV radiation and pollution. Among the key mechanisms driving skin aging, cellular senescence and the depletion of epidermal stem cells play a central role. Senescent cells accumulate over time, creating a pro-inflammatory environment through the senescence-associated secretory phenotype (SASP). SASP factors, including pro-inflammatory cytokines, reactive oxygen species (ROS), and matrix-degrading enzymes, compromise the structural and biochemical integrity of the skin. This disruption of the dermal matrix negatively impacts the regenerative potential of epidermal stem cells, which are essential for maintaining skin homeostasis and repairing damaged tissue.

Epidermal stem cells, located in the basal layer of the epidermis, possess unique self-renewal and differentiation abilities, enabling the daily regeneration of the epidermis. However, with age, these cells undergo functional decline, leading to reduced proliferative capacity, loss of skin elasticity, thinning of the epidermis, and visible signs of aging such as wrinkles and loss of firmness. Preserving the functionality of epidermal stem cells and mitigating cellular senescence are crucial strategies for counteracting these age-related changes.

In this study, we present the development of a new botanical active ingredient derived from the meristematic cells of a resilient coastal plant. Inspired by its natural regenerative capacity, a bioactive extract rich in polyphenols was obtained through biotechnology. The *in vitro* results convincingly demonstrate the extract's ability to mitigate senescence, protect epidermal stem cells, and preserve skin functionality.

2. Materials and Methods

Antioxidant Activity: ROS levels were measured in human epidermal keratinocytes exposed to oxidative stress induced by hydrogen peroxide (H2O2). The extract's ability to reduce ROS was quantified using the DCFH-DA probe.
Anti-inflammatory Activity: Keratinocytes pre-treated with the extract were subjected to inflammation induction using phorbol myristate acetate (PMA). The production of pro-inflammatory cytokines (IL-8 and TNF-α) was measured by ELISA.

Protection of Dermal Matrix Integrity: MMP-1 production was quantified in dermal fibroblasts exposed to UV-induced stress. Collagen structure in human skin explants (55 y.o.) was evaluated through X-PolaR technology to measure birefringence after treatment by topical application for 7 days of the extract in formulation.
Stem Cell Protection: A colony-forming efficiency (CFE) assay was used to evaluate the clonogenic potential of epidermal stem cells under oxidative stress induced by H2O2. The extract's protective effect on stem cell functionality was assessed.
Anti-Senescence Activity: Senescence was induced in dermal fibroblasts using two models: replicative senescence (Hayflick model) and stress-induced premature senescence (SIPS) using chronic oxidative stress. Senescence markers (e.g., beta-galactosidase activity, ROS levels, SASP factors) were evaluated by histochemical staining and ELISA.

3. Results

Several *in vitro* assays were performed to evaluate the anti-aging potential of the botanical extract.

3.1. Antioxidant and anti-inflammatory effects to preserve from environmental stress

The extract dose-dependently and significantly reduced ROS levels in keratinocytes (Figure 1). Given its richness in polyphenols, we compared its efficacy to reference molecules within the same family. At a concentration of 0.1%, its antioxidant efficacy was equivalent to that of quercetin and significantly superior to resveratrol, highlighting the strong antioxidant potential of the botanical extract.

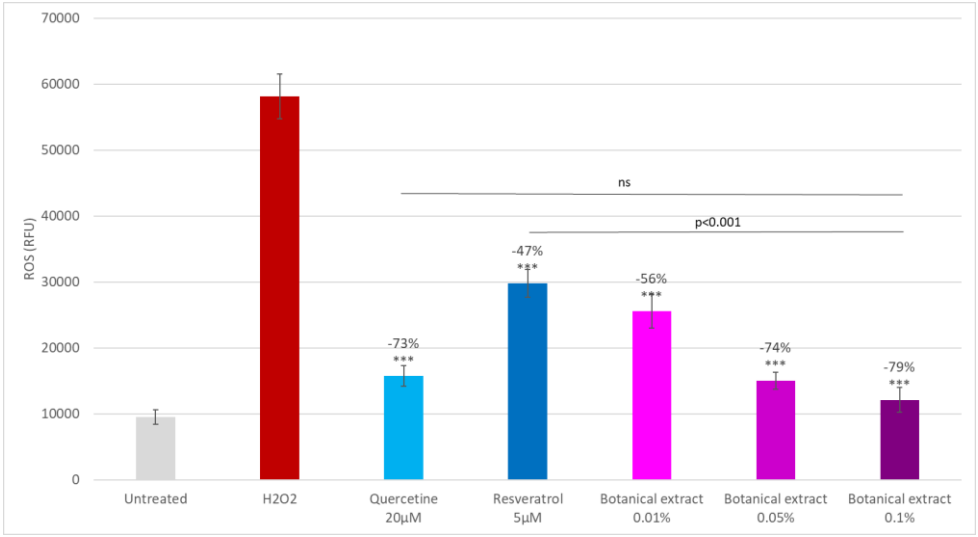

Figure 1. ROS production in NHEK stimulated by H2O2. *Statistical analysis: One-Way ANOVA followed by Dunnet's test: ns non significant; ***p<0.001*.

The anti-inflammatory effect of the extract was demonstrated by the inhibition of pro-inflammatory cytokines TNFα and IL8 release in a PMA-induced model on keratinocytes (Figures 2 & 3).

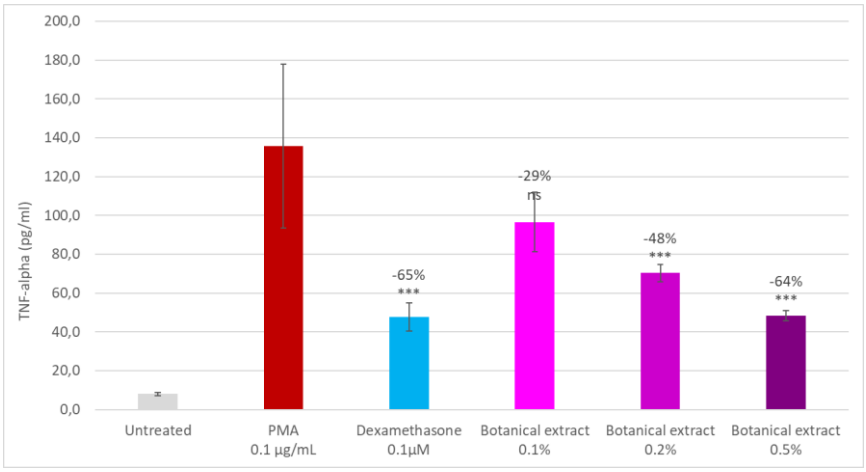

Figure 2. TNFα release in NHEK stimulated by PMA. *Statistical analysis: One-Way ANOVA followed by Dunnet's test: ns non significant; ***p<0.001*.

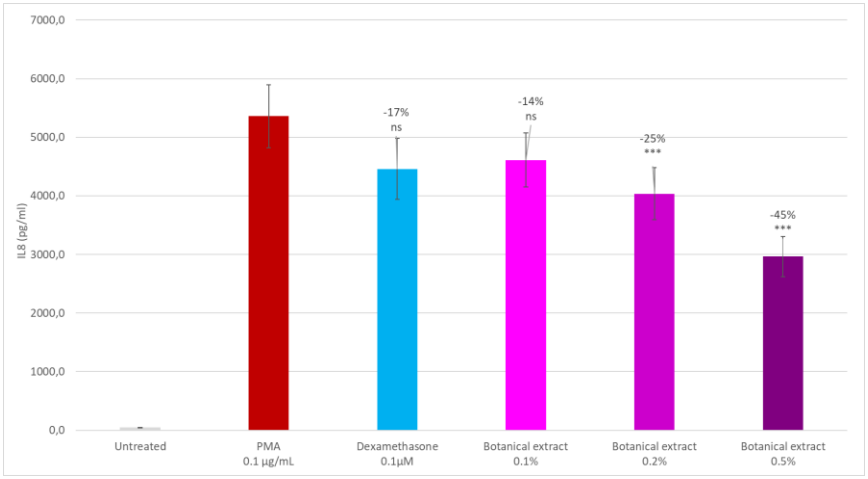

Figure 3. IL8 release in NHEK stimulated by PMA. *Statistical analysis: One-Way ANOVA followed by Dunnet's test: ns non significant; ***p<0.001*.

3.2. Dermal Matrix protection

The botanical extract effectively protected the dermal matrix from UV-induced damage. It significantly reduced MMP-1 secretion in UV-stressed fibroblasts up to -80% (Figure 4).

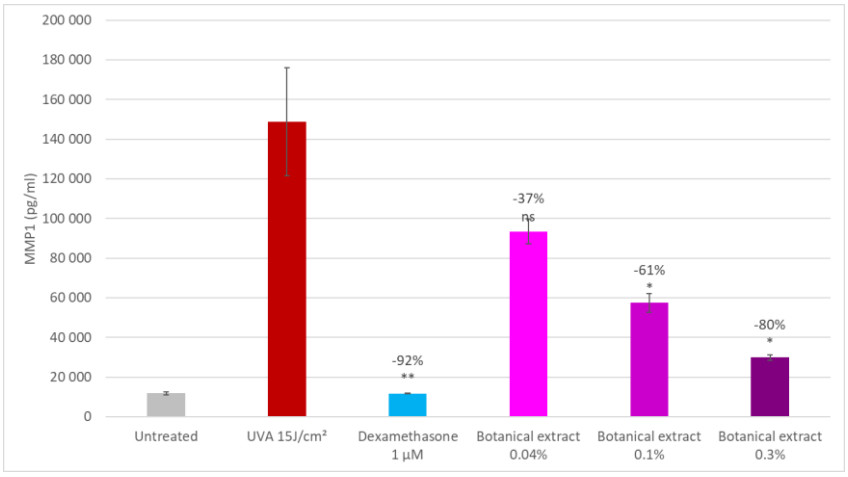

Figure 4. MMP1 release in UVA-irradiated NHDF. *Statistical analysis: Student t test: ns non significant; *p<0.05; **p<0.01*.

The capacity of the botanical extract to preserve the dermal matrix was further evaluated on skin explants by analyzing collagen birefringence using X-PolaR imaging. After 7 days of culture, the Kws parameter significantly decreased, indicating dermal fiber degradation and modeling of aging. Topical application of the botanical extract resulted in a 22% increase in collagen birefringence compared to placebo, suggesting improved collagen organization and dermal matrix integrity (Figure 5).

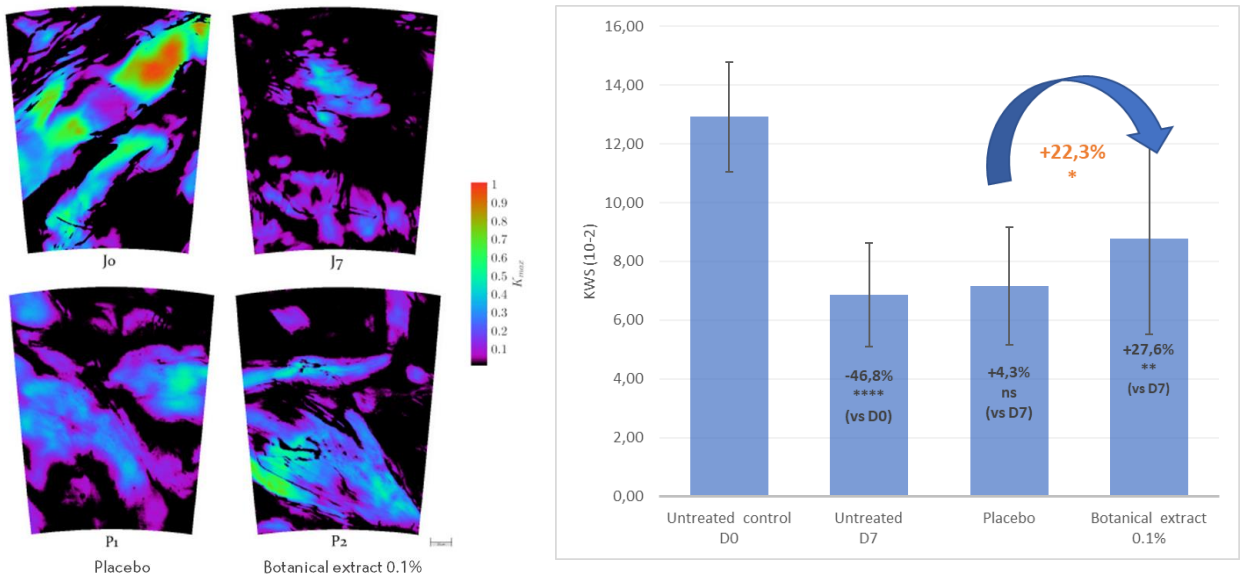

Figure 5. Analysis of the structural state of collagen fibers in the mid-reticular dermis of skin explants using polarization imaging (X-PolaR technology). Measurement of birefringence of the samples, analysis of Kws parameter, which represents the structural quality of the collagen. *Statistical analysis: One-Way ANOVA followed by Dunnet's test: *p<0.05 vs placebo; **p<0.01 vs D7; ****p<0.0001 vs D0*.

3.3. Stem Cell Protection

The extract enhanced the clonogenic potential of epidermal stem cells under oxidative stress. When H2O2 was applied, a 78% decrease in colony-forming efficiency (CFE) was observed (Figure 6A), while treatment with the extract restored CFE by 48% (Figure 6B), demonstrating its capacity to preserve stem cell functionality.

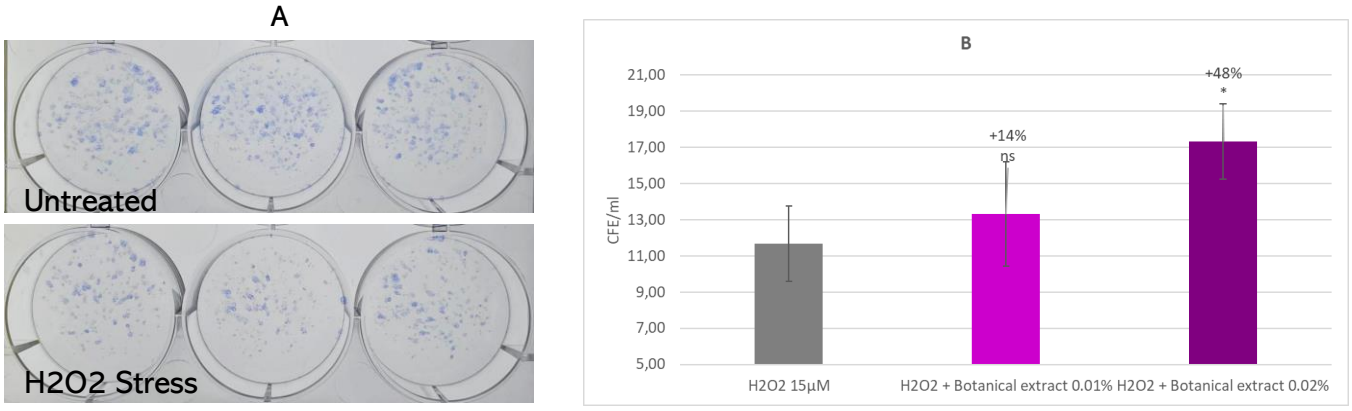

Figure 6. Analysis of the clonogenic potential of epidermal progenitor cells under H2O2 stress: colony forming efficiency (CFE) assay. A) Representative pictures of epidermal stem cells clones, impact of H2O2 stress; B) CFE quantification, protective effect of the extract toward H2O2 stress. *Statistical analysis: Student t test: ns non significant; *p<0.05.*

3.4. Anti-Senescence Effects

The extract exhibited senomorphic properties in two models of senescence in dermal fibroblasts:

In the replicative senescence model (Hayflick), the extract reduced SASP factors, with a 38% decrease in IL-8 and 26% reduction in MMP-1 secretion (Figure 7).

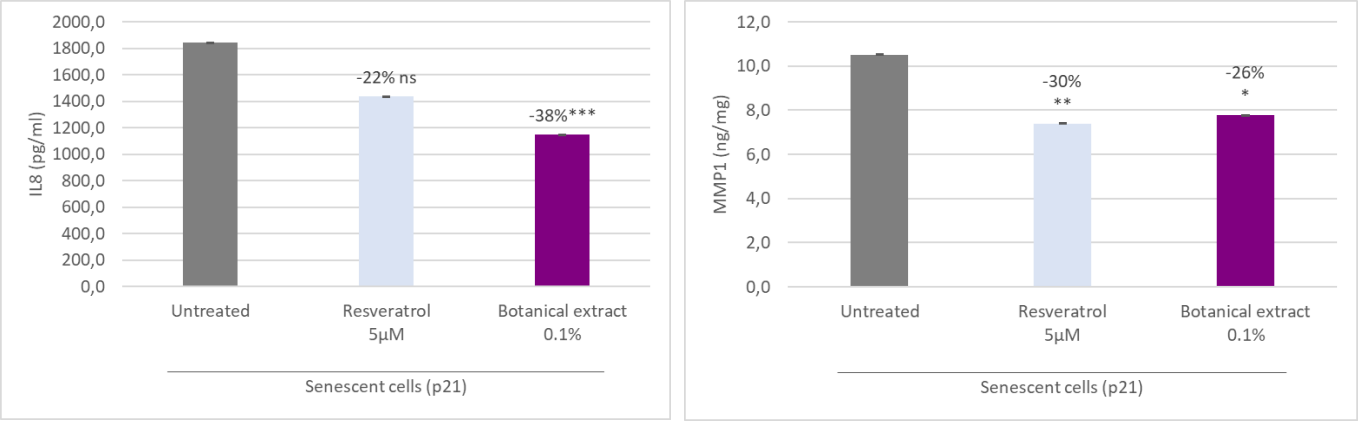

Figure 7. Senescence-Associated Secretory Phenotype (SASP) analysis in dermal fibroblasts brought to senescence by replication (over 21 passages – Hayflick model): ELISA quantification of IL8 and MMP1. *Statistical analysis: Student t test: ns non significant; *p<0.05; **p<0.01; ***p<0.001.*

In a model of Stress-Induced Premature Senescence (SIPS) caused by repetitive H2O2 stress, the expression of the senescence marker SA-β-Gal was strongly increased, confirming the senescence phenotype. This was accompanied by an increase in SASP factors IL-8 and ROS, as well as a decrease in collagen-1. The botanical extract mitigated oxidative stress-induced senescence by significantly reducing beta-galactosidase activity by 62%, restoring pro-collagen I levels by 48%, reducing ROS by 43%, and decreasing IL-8 levels by 37% (Figure 8).

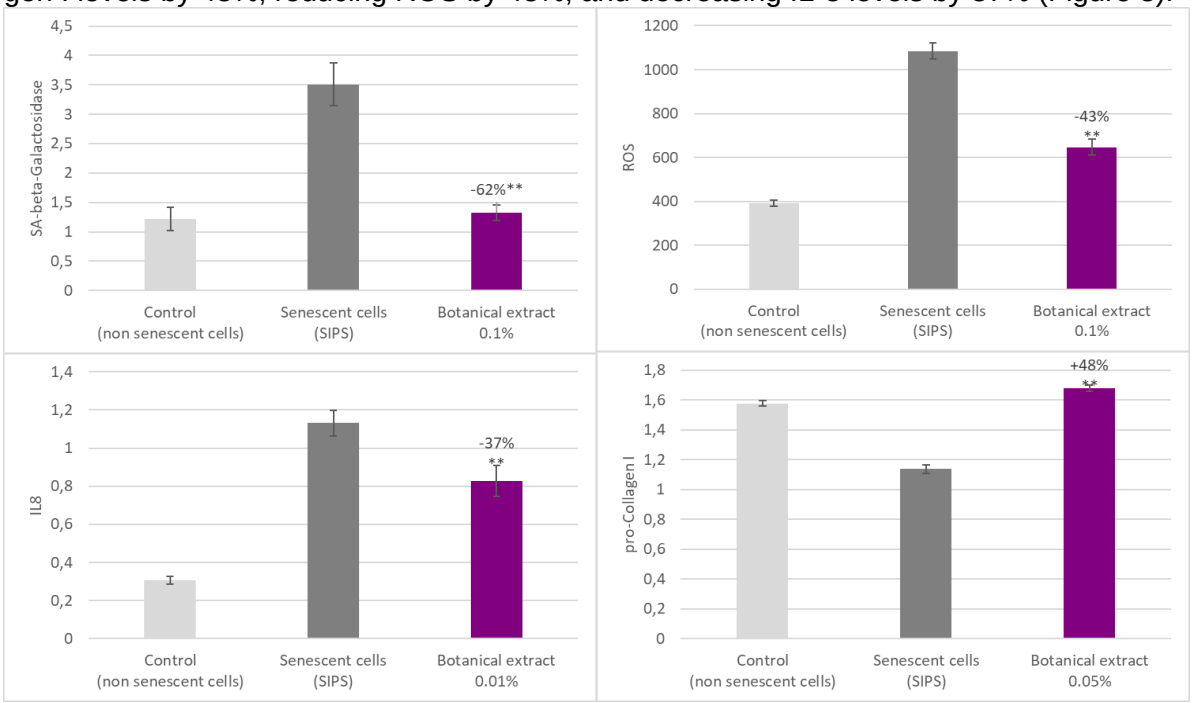

Figure 8. Expression of SA-β-Galactosidase, SASP factors IL8 and ROS, as well as pro-collagen I in dermal fibroblasts brought to senescence by SIPS (chronic oxidative stress). *Statistical analysis: Student t test: **p<0.01.*

4. Discussion

These findings align with previous research emphasizing the importance of antioxidants in combating skin aging. The study suggests that the botanical extract, with its potent antioxidant activity, could serve as a valuable component in the development of advanced anti-aging formulations, offering a sustainable strategy for improving skin resilience and vitality over time. The study highlights the significant potential of a new botanical extract in addressing the complex mechanisms underlying skin aging. The extract's ability to preserve epidermal stem cells and reduce cellular senescence is particularly noteworthy, as these factors play a crucial role in maintaining skin health and vitality. The antioxidant and anti-inflammatory properties of the extract further contribute to its efficacy by protecting the skin from oxidative damage and inflammation, which are key contributors to the aging process.

The findings align with previous research that emphasizes the importance of targeting cellular senescence and stem cell functionality to combat skin aging. By mitigating the effects of senescence-associated secretory phenotype (SASP) factors and preserving the dermal matrix integrity, the extract offers a comprehensive approach to enhancing skin longevity. The study's results suggest that this botanical extract could serve as a valuable component in the development of advanced anti-aging formulations.

5. Conclusion

This study demonstrates the promising potential of a novel botanical extract in targeting key biological mechanisms of skin aging. By protecting epidermal stem cells, mitigating cellular senescence, and preserving dermal matrix integrity, this active ingredient represents a promising approach for advancing anti-aging treatments. The multi-targeted actions of the extract provides a new way for promoting skin longevity, emphasizing the importance of maintaining cellular health and regenerative capacity to combat visible and structural signs of aging. This innovative approach could offer a more sustainable strategy for improving skin resilience and vitality over time.