

Targeting photoaging through unsaponifiable-enriched grape seed oil: innovative insights into solar-induced skin protection

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INTRODUCTION

Solar radiation includes UV, blue light, and infrared (IR) wavelengths, each contributing differently to skin damage. UVA (320–400 nm) penetrates deeply into the dermis and is a major source of oxidative stress, generating ROS that trigger irreversible protein carbonylation and structural deterioration (1). Blue light (380–500 nm) from natural and artificial sources also boosts ROS production, accelerating protein oxidation, skin aging, and barrier weakening (2). IR (>760 nm) reaches the deepest layers, inducing thermal stress and oxidative damage to key macromolecules such as collagen I and filaggrin, reducing firmness and compromising barrier function (3). Together, these wavelengths drive DNA damage, inflammation, MMP activation, reduced collagen synthesis, and ultimately photoaging (4).

This study evaluates a grape seed oil enriched (GSOE) in unsaponifiable compounds to determine its ability in mitigating solar radiation-induced skin damage.

MATERIALS & METHODS

1/ Tocopherols, tocotrienols and vitamin E contents analysis

A liquid chromatography method with UV detection was used to quantify tocopherols, tocotrienols, and total vitamin E in samples. Standard and test solutions were prepared in hexane/isopropanol and analyzed under isocratic conditions on a Lichrospher Si 60 column. Quantification was based on peak areas, using bracketing standards and validated through repeatability (RSD ≤ 2%). Total vitamin E content was obtained by summing tocopherol and tocotrienol fractions from duplicate test solutions.

2/ In vitro analysis of interleukin-6 secretion under thermal stress

Keratinocytes were seeded at the density of 9000 cells/well in a 96-well plate. After 48 hours, cells were treated with Grape seed oil enriched at 0.001%. After 24 hours of treatment, cells were stressed during 30 minutes at 45°C in a water bath and incubated for 24h à 37°C. Supernatants were collected, and IL-6 concentration was assayed using the Human IL-6 Quantikine ELISA kit

3/ Protein carbonylation analysis by immunofluorescent on human skin explants

Explants were topically treated with the test products (2 mg/cm²) and, 24 hours later, exposed to UVA, blue light, or infrared using the OxiProteomics® irradiation system. After irradiation, explants were transferred to fresh medium and collected three hours later, then embedded in OCT. Carbonylated proteins were labeled with a specific fluorescent probe alongside DAPI for nuclear staining.

| Table 1. Experimental Groups |                                                             |          |
|------------------------------|-------------------------------------------------------------|----------|
| Description                  | Treatment                                                   | Sampling |
| Control                      | Not treated, Not Stressed                                   | 3h       |
| Blue Light Stress            | Not treated + Blue Light irradiation (λ=460 nm; 14.4 J/cm²) |          |
| Placebo + Stress             | Treated with product (2 mg/cm²)                             |          |
| GSOE + Stress                | Blue Light irradiation (λ=460 nm; 14.4 J/cm²)               |          |
| UV-A Stress                  | Not treated + UV-A irradiation (λ=365 nm; 6 J/cm²)          |          |
| Placebo + Stress             | Treated with product (2 mg/cm²)                             |          |
| GSOE + Stress                | UV-A irradiation (λ=365 nm; 6 J/cm²)                        |          |
| Infrared Stress              | Not treated + Infrared irradiation (λ=850 nm; 42/43°C)      |          |
| Placebo + Stress             | Treated with product (2 mg/cm²)                             | 24h      |
| GSOE + Stress                | Infrared irradiation (λ=850 nm; 42/43°C)                    |          |

4/ Immunofluorescence analysis of protein carbonylation in human skin explants

Explants were topically treated with the test products (2 mg/cm²) and, 24 hours later, exposed to infrared irradiation (850 nm; 42–43 °C). Twenty-four hours after IR stress, samples were embedded in OCT. For marker detection, 5-µm cryosections were prepared, blocked and incubated with primary antibodies targeting filaggrin or collagen I. Sections were then washed and imaged for quantitative analysis.

| Table 2. Experimental Groups |                                                        |          |
|------------------------------|--------------------------------------------------------|----------|
| Description                  | Treatment                                              | Sampling |
| Control                      | Not treated, Not Stressed                              | 24h      |
| Infrared Stress              | Not treated + Infrared irradiation (λ=850 nm; 42/43°C) |          |
| Placebo + Stress             | Treated with product (2 mg/cm²)                        |          |
| GSOE + Stress                | Infrared irradiation (λ=850 nm; 42/43°C)               |          |

RESULTS

1/ Tocopherols, tocotrienols and vitamin E contents analysis

Our analyses revealed a strong enrichment for all three compounds, with concentrations increased by 8.73-fold for vitamin E, 8.74-fold for tocopherols, and 8.71-fold for tocotrienols compared with the non-enriched grape seed oil.

| Compounds    | Grape seed oil  | Grape seed oil enriched | Concentration factor |
|--------------|-----------------|-------------------------|----------------------|
| Vitamin E    | 46.35 mg/100 gr | 404.4 mg/100 g          | 8.73                 |
| Tocopherols  | 23.05 mg/100 g  | 201.40 mg/100 g         | 8.74                 |
| Tocotrienols | 23.30 mg/100 g  | 203 mg/100 g            | 8.71                 |

Table 3: Quantification of the compounds before and after the enrichment process

2/ In vitro analysis of interleukin-6 secretion under thermal stress

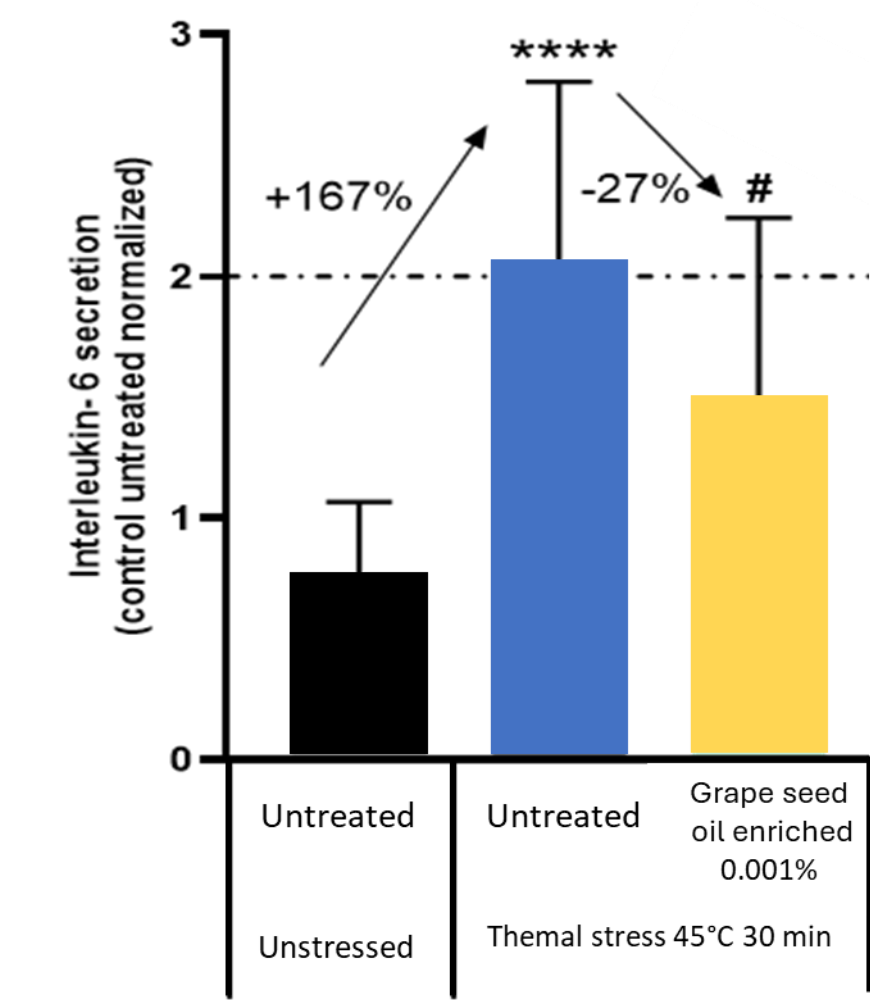


Figure 1. Quantification of Interleukin-6 on normal human keratinocytes after IR stimulation

As expected, thermal exposure induced a significant increase in IL-6 production, reaching +167% compared with the basal condition (p < 0.0001). Pre-treatment with our active ingredient reduced IL-6 secretion by 27% relative to the stressed group (p < 0.05), demonstrating its anti-inflammatory effect in this thermal stress model.

3/ Protein carbonylation analysis by immunofluorescent on human skin explants

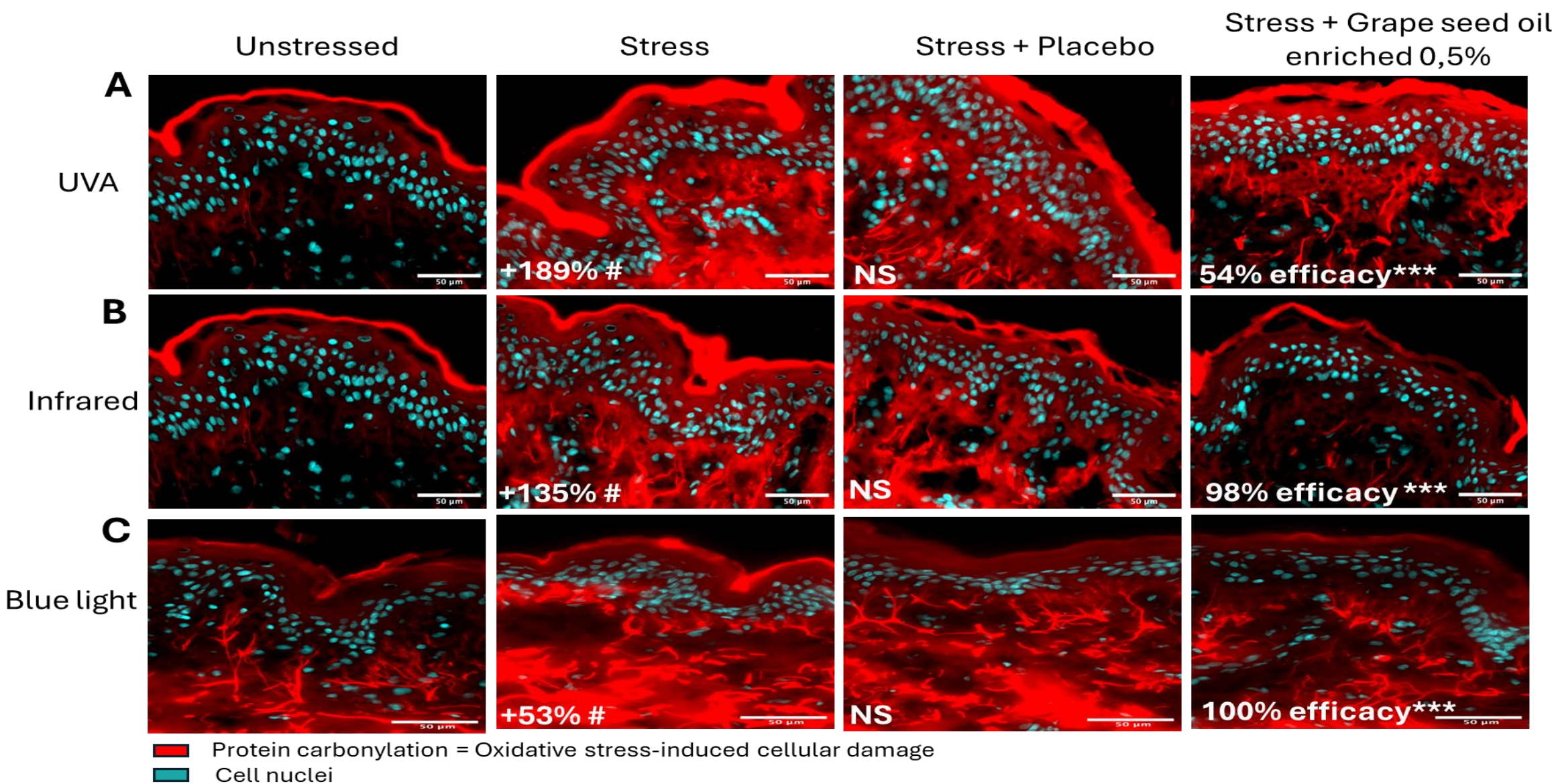


Figure 2. Quantification of carbonylation levels after stress ex vivo. A) UVA stress, B) Infrared stress, and C) Blue light stress. \*\*\* p<0.001; one-way ANOVA and Dunnett’s post-hoc test for multi-comparisons vs Stress group (alpha=0.05), # p<0.001 (vs unstressed group).

4/ Immunofluorescence analysis of protein carbonylation in human skin explants

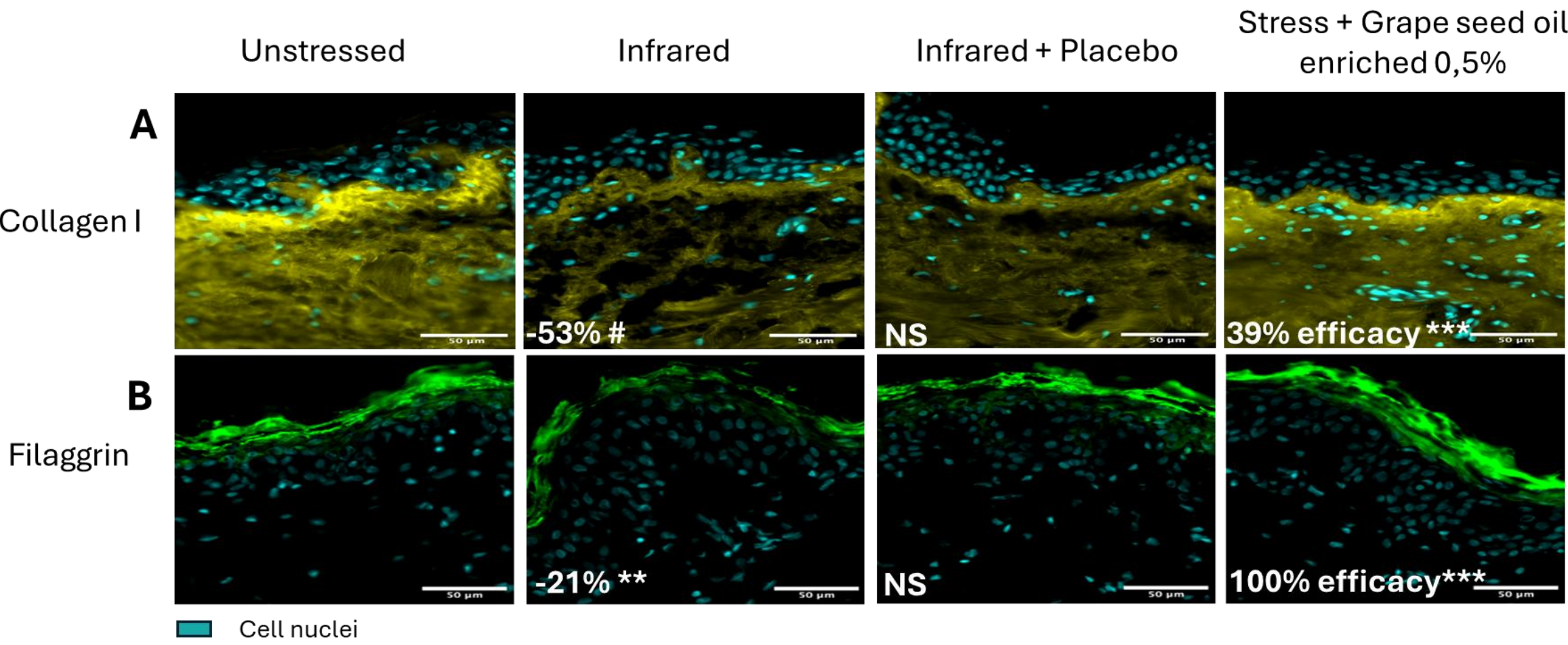


Figure 3. In situ visualization of collagen I and filaggrin levels by epifluorescence microscopy. A) Collagen I levels and B) Filaggrin levels of each experimental group are expressed as relative values (% vs Control). \*\* p<0.01, \*\*\* p<0.001, one-way ANOVA and Dunnett’s post-hoc test for multi-comparisons vs Stress group (alpha=0.05) and # p<0.001 (vs unstressed group).

CONCLUSION

Overall, these data show that the grape seed oil enriched acts through multiple complementary mechanisms: it protects against oxidative stress, preserves essential skin structures, and reduces inflammation. Together, these effects position the extract as a highly relevant ingredient for preventing photoaging induced by UVA, IR, and blue light, and for maintaining the integrity and youthfulness of skin exposed to solar-related aggressions.

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