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"Bioinspired Homeostasis Protection: Hacking the Power of Raspberry Leaves Ellagitannins to Target Hallmarks of Aging"

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1. Introduction

Skin aging is driven by the gradual breakdown of cellular homeostasis under both intrinsic and extrinsic influences. Among the biological processes involved, mitochondrial dysfunction is now recognized as a central hallmark of aging [1-3]. It includes impaired energy production, increased reactive oxygen species (ROS) generation, defective organelle turnover, and destabilization of respiratory chain complexes—events implicated in senescence, inflammation, and tissue degeneration [4-5].

Beyond energy metabolism, mitochondria also act as sensors of mechanical and metabolic signals, influencing cell fate through complex signaling. Their progressive decline with age contributes to oxidative stress, proteostasis imbalance, and reduced regenerative capacity [5-6]. In skin, these changes manifest as decreased cell proliferation, extracellular matrix degradation, and impaired communication, leading to skin tissue atrophy, wrinkle formation, and pigmentary disorders [1,6].

As the body's outermost barrier, the skin is continuously exposed to UV radiation, pollution, and microbial challenges. Maintaining a stable redox balance—conceptualized as the skin redoxome—is essential for epidermal and dermal integrity. Its disruption promotes oxidative stress, inflammation, and pigmentation irregularities [7-8].

Mitochondrial dynamics, including fusion, fission, and cytoskeletal interactions, are highly sensitive to mechanical forces and feed back into redox regulation and metabolic pathways [4-5]. Mitochondrial dysfunction, thus, is a key intersection between mechanotransduction, energy metabolism, and oxidative stress, making it an attractive target for anti-aging strategies [9].

In this context, bioinspired approaches based on plant resilience mechanisms are of growing interest. Long-lived plants have evolved sophisticated antioxidant systems to protect cellular integrity under stress. Ellagitannins (ETs) contribute to this defense by limiting oxidative damage and preserving organelles such as mitochondria [10-11].

Here, we investigated an eco-designed ETs-rich extract obtained from raspberry leaf biomass using an innovative subcritical water-glycol co-extraction. We hypothesized that this extract could reinforce cellular longevity by preserving mitochondrial structure, enhancing proteostasis, and mitigating senescence. Our objective was to assess its multi-targeted protective effects through a combination of molecular, cellular, and clinical approaches, with the aim of demonstrating that supporting mitochondrial and redox homeostasis is a viable strategy for maintaining skin function and resilience over time.

2. Materials and Methods

2.1. Proteomic Analysis

Human Skin Fibroblasts (HSFs, ATCC® CRL-2106™) were exposed to tert-butyl hydroperoxide (tBHP, 5 µM) and treated at the same time with ETs-rich extract (0.3%) for 72h, n=3.

Protein extraction was performed after cell lysis using RIPA buffer (Sigma-Aldrich), and protein concentration was determined by BCA assay (Thermo Fisher Scientific).

Proteins were analyzed by nano-liquid chromatography-tandem mass spectrometry (nLC-MS/MS), as described in [11]. Data were processed using appropriate bioinformatics pipelines, and pathway enrichment was conducted via Ingenuity Pathway Analysis (IPA, Qiagen). For each pathway (node, circle), the abundance of the cluster is represented by a color scale (Figure 1) relative to its control (ratio). Node size indicates the number of proteins in the cluster, and significant clusters are shown.

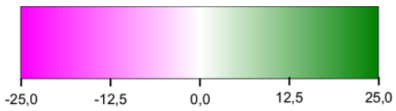


Figure 1. Network Scale: increase in green; decrease in magenta; white, no change)

2.2. Mitochondrial Network Integrity

HSFs (ATCC® CRL-2106™) were cultured and pretreated with ETs-rich extract (0.15%) for 48h, followed by UVA irradiation (10 J/cm², 340–400 nm spectrum) for 10 minutes.

Mitochondria and nuclei were stained with MitoTracker™ Red CMXRos and DAPI, respectively, and imaged using BioTek Cytation (Agilent).

Quantitative analysis of mitochondrial morphology (network length, branching, fragmentation) was performed using ImageJ with the 'MitoAnalyzer' plugin; n=21/24 cells – distributed in 3 separate experiments.

2.3. Clinical Evaluation of Anti-aging Efficacy

The anti-aging efficacy of the ETs-rich extract was evaluated in a double-blind, placebo-controlled trial over 56 days in 66 women aged 40–70 years. Volunteers applied twice daily either a ETs-rich extract cream (3%) or a placebo to the face and on a selected dark spot (face, neckline, shoulders, or hands).

Facial wrinkles, and pigmentation homogeneity were assessed by image analysis performed by specific software (Spincontrol) from standardized pictures taken with the VISIA®-CR system (Canfield) at baseline and after treatment: crow's feet and under-eye wrinkles (topographic parameter), and pigmentary irregularities were quantified. Dark spot improvement was evaluated by colorimetric analysis (ITA° and pigmentation index) using the C-Cube system (Pixience).

2.4. Statistical Analysis

Statistical analyses were adapted to each experimental model. For proteomic profiling, the Ingenuity Pathway Analysis (IPA) 'Core Analysis' tool was employed to identify relevant biological relationships, functions, and pathways (significant clusters are shown $p \leqslant 0.05$). For mitochondrial network morphology assessments, as normality could not be confirmed, a Kruskal-Wallis test followed by Dunn's multiple comparisons test was applied. In the clinical study, intra-group comparisons between baseline (D0) and post-treatment (D56) values were analyzed using paired t-tests, while inter-group comparisons were performed using Student's t-test for independent samples. Statistical significance was denoted as follows in legends:
#$p \leqslant 0.1$, *$p \leqslant 0.05$, **$p \leqslant 0.01$, ***$p \leqslant 0.001$, and ****$p \leqslant 0.0001$.

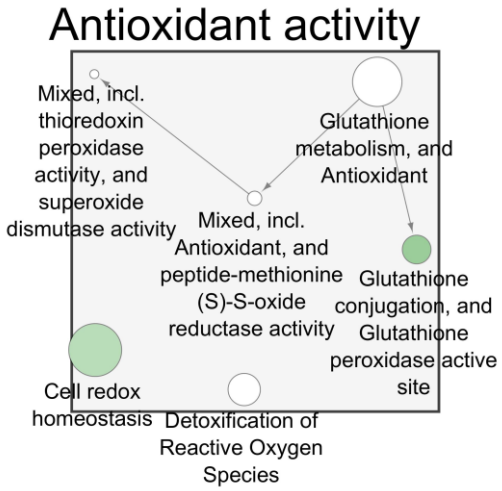
3. Results

3.1. Reinforcement of Redox Homeostasis, Proteostasis, and DNA Repair Mechanisms under Oxidative Stress

Proteomic profiling of fibroblasts exposed to tBHP oxidative stress revealed significant modulations across stress response pathways. Notably, tBHP significantly disrupts mitochondrial homeostasis, as evidenced by a marked downregulation of proteins involved in mitochondrial gene expression (-8%, $p<0.05$) and translation (-9%, $p<0.05$), not shown. This suggests that tBHP impairs mitochondrial protein synthesis and energy production, indicative of mitochondrial dysfunction and oxidative stress. In response, fibroblasts activate adaptive antioxidant defenses, characterized by increased glutathione conjugation and peroxidase activities (+10%, $p<0.01$), consistent with the role of glutathione as a key intracellular antioxidant [1,12] (Figure 2a).

Moreover, tBHP exposure triggered endoplasmic reticulum (ER) stress (Figure 2b), as shown by increased unfolded protein response (UPR, +5% and +6%, both $p<0.05$) and aggrephagy markers (+5%, $p<0.05$), indicating elevated proteotoxic stress and activation of cellular defense mechanisms.

Exposure to tBHP also significantly altered key cellular processes involved in cell cycle progression (not shown) and DNA repair (Figure 2c). Proteomic analysis revealed strong downregulation of mismatch repair (–29%, p<0.0001) and nucleotide excision repair (NER, –23%, p<0.0001) pathways, critical for maintaining genomic stability. Additionally, proteins involved in mitosis, including cytokinesis, nuclear division, and the G1/S transition, were markedly reduced, indicating cell cycle arrest and supporting the induction of a senescent phenotype (not shown).



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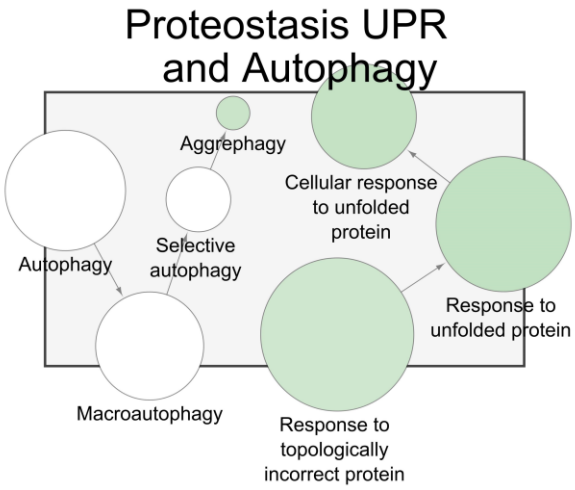

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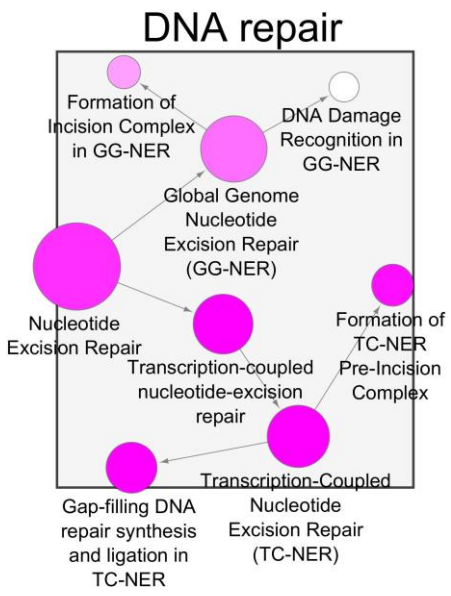


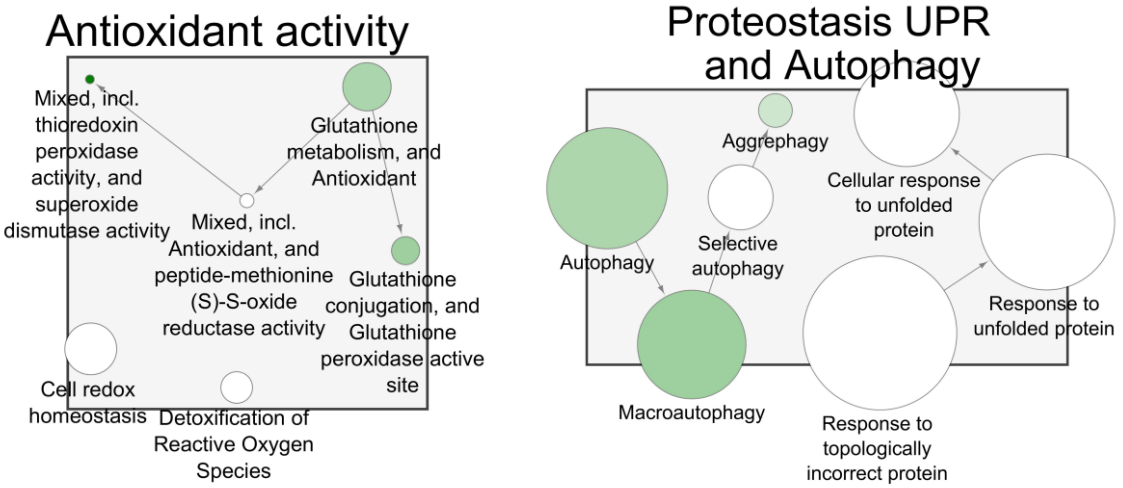
(c)

Figure 2. Functional protein interaction network of gene expression based on available annotation after tBHP treatment (ratio *versus* non-treated control): (a) antioxidant activity; (b) proteostasis and autophagy; (c) DNA repair canonical pathways.

Treatment with the ETs-rich extract demonstrated a protective effect against tBHP-induced oxidative stress. Specifically, while glutathione pathway activation remained high (+9%, p<0.01 *vs* tBHP alone), the extract also boosted additional antioxidant systems (Figure 3a): thioredoxin peroxidase and superoxide dismutase activities increased significantly (+26%, p<0.01),

suggesting enhanced reactive oxygen species (ROS) detoxification capacity and an important role in maintaining redox homeostasis.

The ETs-rich extract further enhanced autophagic responses, indicating restoration of proteostasis. While proteins involved in the UPR remain stable under ETs-rich extract treatment, general autophagic mechanisms are significantly increased (Figure 3b): macroautophagy (+10%, p<0.01), autophagy (+8%, p<0.01), and aggrephagy (+5%, p<0.05).



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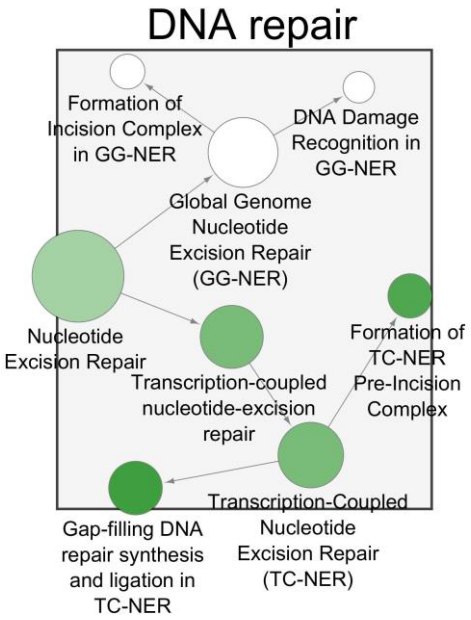


(c)

Figure 3. Functional protein interaction network of gene expression based on available annotation after ETs-rich extract treatment at 0.3% (ratio *versus* tBHP stress): (a) antioxidant activity; (b) proteostasis and autophagy; (c) DNA repair canonical pathways.

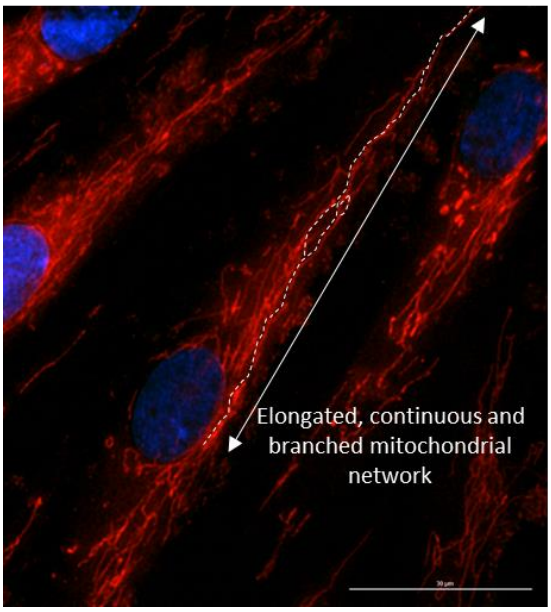
Regarding cell cycle progression and DNA repair capabilities, treatment with the ETs-rich extract attenuated the tBHP-induced senescent phenotype. Notably, Transcription-Coupled Nucleotide Excision Repair (TC-NER) protein levels, which were down-regulated by tBHP (-28%, -25%, both p<0.0001 and -24%, p<0.001, Figure 3c), were restored (13%, 17%, both p<0.05 and 19%, p<0.01, Figure 2c), suggesting a recovery of DNA repair capacity. Similarly, proteins

involved in late cytokinesis stages showed increased abundance, indicating partial reactivation of mitotic processes (not shown).

Overall, these findings highlight that the ETs-rich extract mitigates oxidative stress, preserves mitochondrial and proteostatic functions, and supports DNA repair and cell cycle progression, ultimately contributing to cellular homeostasis maintenance and skin cell longevity.

3.2. Mitochondrial Network Protection

In healthy fibroblasts, the mitochondrial network appears elongated, continuous, and highly branched (Figure 4a), reflecting dynamic fusion and fission processes essential for energy distribution and organelle maintenance [5].



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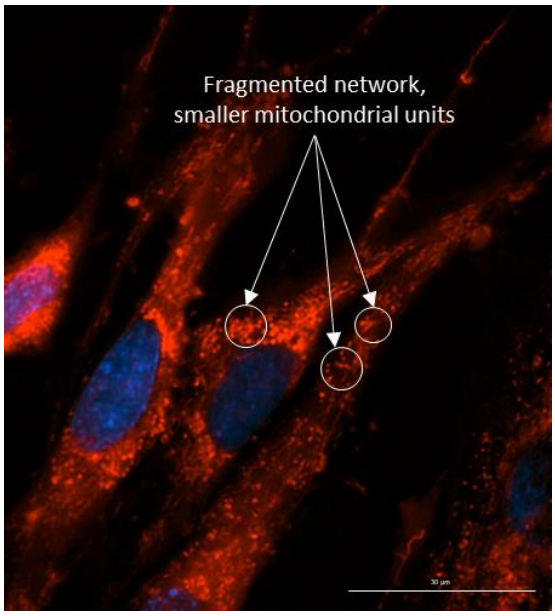

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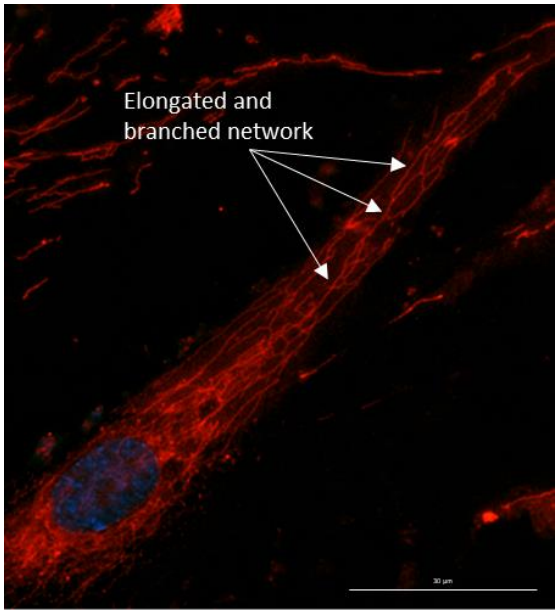

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Figure 4. Visualisation of fibroblasts mitochondrial network (red) and nuclei (blue): (a) non-treated control; (b) UVA (10 J/cm²); (c) ETs-rich extract at 0.15% + UVA.

Upon UVA irradiation, a marked disruption of mitochondrial morphology was observed, characterized by extensive fragmentation and a significant reduction in network length (Figure 4b). Mitochondria appeared as small, isolated units with diminished cytoplasmic distribution, indicating impaired mitochondrial fusion and early signs of dysfunction.

Pretreatment with the ETs-rich extract significantly preserved mitochondrial architecture under UVA-induced stress. Cells co-treated with the extract maintained elongated, interconnected mitochondrial networks similar to non-irradiated controls (Figure 4c). These findings suggest that the ETs-rich extract helps protect mitochondrial integrity, supporting energy homeostasis and enhancing cellular resilience against photoaging-associated oxidative damage.

Mitochondrial network morphology was evaluated by multiparametric single-cell analysis (Table 1). UVA exposure led to an increase in mitochondrial particle number compared to non-treated control (54%, $p<0.01$) and, by a decrease in particle length (31%, $p<0.0001$) and a reduction in network branching (17%, $p<0.01$). These alterations are consistent with mitochondrial fragmentation and loss of structural integrity.

Treatment with the ETs-rich extract significantly preserved mitochondrial morphology. Compared to UVA alone, the extract reduced mitochondrial particle number by 49%, improved mean particle length by 31% (both $p<0.0001$), and restored network branching (+17%, $p<0.1$).

Table 1. Evaluation of mitochondrial network integrity by multiparametric single-cell analysis.

Parameter (± SD)	**Non treated control**	**Control + UVA (10J/cm²)**	**ETs-rich extract (0.15%) + UVA (10J/cm²)**
Mitochondrial particles number/cell	**122.1** ± 53.8	**188.4** ± 64.9	**95.9** ± 40.9
Mitochondrial particles lenght/cell (µm)	**2.984** ± 0.450	**2.054** ± 0.329	**2.681** ± 0.450
Mitochondrial branching/cell (mean)	**4.674** ± 0.671	**3.864** ± 0.732	**4.503** ± 0.805

These results indicate that the ETs-rich extract effectively preserves mitochondrial network organization and limits UVA-induced mitochondrial network disruption.

3.3. Improvement of Skin Wrinkles and Pigmentation Homogeneity

After 56 days of twice-daily application, treatment with the 3% ETs-rich extract cream led to significant improvements in visible signs of aging compared to placebo, as assessed by standardized image analysis (Figure 5). Wrinkle surface area in the periorbital region decreased significantly in the ETs-rich extract group (–6.7%, $p<0.01$), while the placebo group showed no significant change (+0.9%, ns). A significant difference in favor of the ETs-rich extract was observed, indicating a superior anti-wrinkle effect ($p<0.01$).

Regarding skin pigmentary disorders, the ETs-rich extract induced a significant reduction in pigmentation heterogeneity (–10.6%, $p<0.001$), whereas placebo treatment had no significant effect (–0.5%, ns). A significant difference in favor of the ETs-rich extract was observed, indicating an enhanced superior anti-wrinkle effect (-7.6%, $p<0.01$). Colorimetric analysis of dark

spots revealed that both treatments significantly improved skin color parameters (ITA° and melanin index, Figure 6). However, the ETs-rich extract led to a greater enhancement, with a +37.4% increase in ITA° and a –7.1% reduction in melanin index (both $p<0.001$), highlighting its superior efficacy in improving skin tone and reducing pigmentation irregularities ($p<0.1$).

Together, these results confirm that the ETs-rich extract effectively reduces wrinkles, and enhances pigmentation homogeneity after 56 days of use.

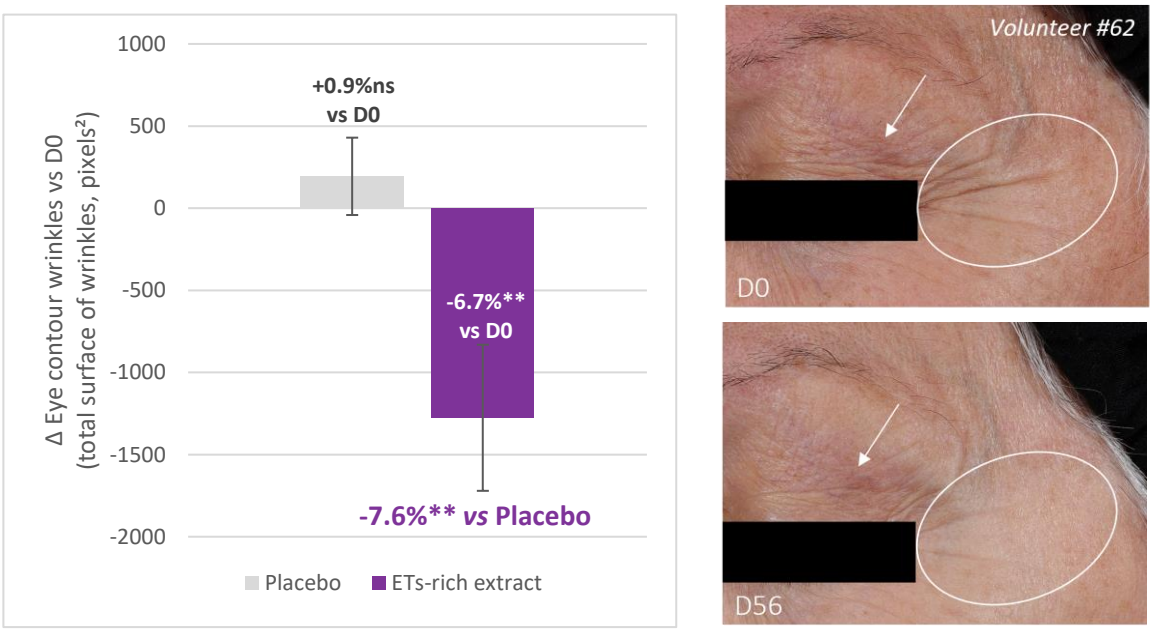

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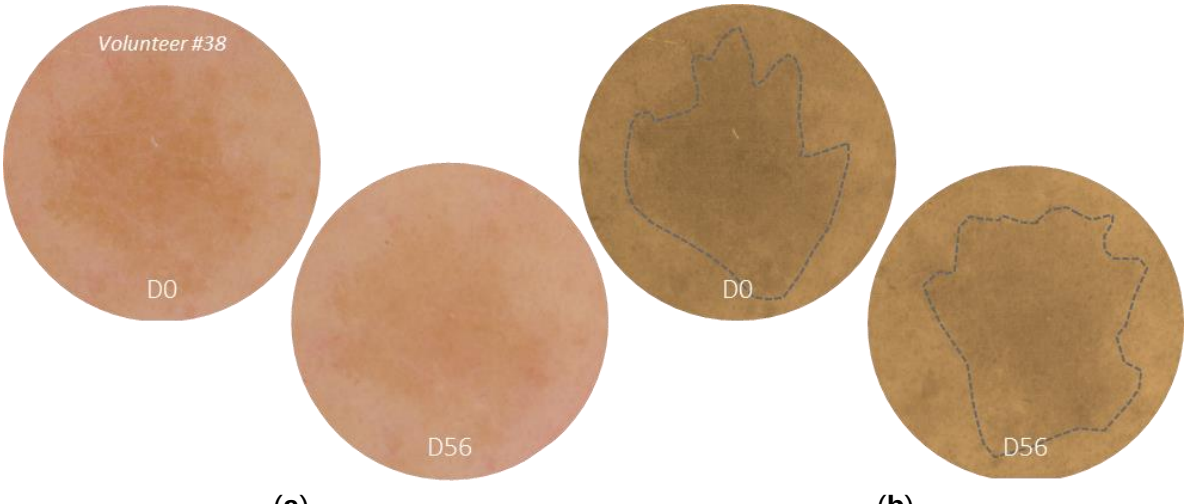
Figure 5. Wrinkles surface of crow's feet (circles) and undereye (arrows) regions: (a) Eye contour wrinkles variation versus D0 (pixels²); (b) VISIA®-CR illustrations for the volunteer #62 at the baseline (D0) and after 56 days of twice-daily application of the active cream.



(**a**) (**b**)

Figure 6. C-CUBE® illustrations of a selective dark spot on the face for the volunteer #38 at the baseline (D0) and after 56 days of twice-daily application of the active cream: (a) macrodermoscopy; (b) melanin index filter.

4. Discussion

Maintaining mitochondrial network integrity is essential for sustaining mitochondrial function, cellular energy production, and tissular redox homeostasis [4,8]. Disruptions in these pathways are known to contribute to increased ROS production, reduced cellular performance, and the progression of skin aging [2,4]. In this study, treatment with the ETs-rich extract preserved mitochondrial morphology under UVA-induced stress, maintaining network connectivity and structural organization. This preservation likely supported the functional improvements observed in cell renewal and viability. Proteomic analysis further confirmed the activation of redox-regulating enzymes, aligning with established roles of antioxidant responses in maintaining skin homeostasis [12-14].

Although mechanisms such as the unfolded protein response (UPR) and autophagy aim to maintain protein quality by limiting the accumulation of misfolded proteins [14-15], persistent stress can overwhelm these systems, leading to cell dysfunction and senescence [16]. The ETs-rich extract enhanced autophagic clearance pathways, suggesting its role in supporting proteome stability and limiting long-term proteotoxic damage.

Alterations in DNA repair and cell cycle progression are strongly associated with the induction of cellular senescence [12,17-19]. Accumulation of senescent cells contributes to reduced skin regenerative capacity and tissue deterioration over time [18-19]. The ETs-rich extract restored DNA repair activities and improved mitotic dynamics. These findings were supported by additional *in vitro* experiments (not shown) showing a reduction in H_2O_2-induced senescence and preservation of keratinocyte clonogenicity under UV exposure.

Altogether, the extract supported key cellular defense systems involved in mitochondrial maintenance, genomic integrity, and protein homeostasis—mechanisms closely associated with tissue regeneration and skin longevity [1-3,19]. Clinically, its application significantly improved wrinkle appearance and pigmentation uniformity, indicating its relevance for protecting against visible signs of aging while supporting overall skin resilience.

5. Conclusion

This study demonstrates that the ETs-rich extract exerts broad protective effects on biological mechanisms central to skin aging, including mitochondrial function, redox balance, proteostasis, DNA repair, and cell cycle regulation. By preserving mitochondrial network integrity and enhancing antioxidant and autophagic responses, the extract helps maintain cellular homeostasis under stress conditions. These effects contribute to a reduction in senescence, support regenerative capacity, and align with the clinical improvements observed in skin wrinkles and pigmentation uniformity. Together, the results support the relevance of targeting mitochondrial dynamics and stress adaptation pathways to preserve skin health. Bioinspired strategies such as this offer promising avenues to counteract the multifactorial processes of aging.

6. References

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