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Helianthus Annuus Sprout Extract as a Promising Ingredient for Mitochondrial Protection in Sunscreen Formulation

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1. Introduction

Prolonged exposure to solar ultraviolet (UV) rays is a significant contributor to skin aging, with studies indicating that up to 80% of skin aging can be attributed to such exposure [1,2]. This environmental stressor not only accelerates the visible signs of aging, such as wrinkles and pigmentation, but also induces cellular toxicity, leading to cell death and various skin disorders including acute skin burns [3]. UV exposure penetrates subcutaneous tissues, heats blood and deeper tissues, and damages collagen type I, ultimately contributing to photoaging. Symptoms of photoaging include reduced skin elasticity, firmness, and density. Central to these processes is mitochondrial dysfunction, which plays a pivotal role in the cellular response to UV radiation [4].

Mitochondria are essential organelles responsible for energy production through ATP synthesis and are integral in regulating apoptosis, oxidative stress, and various signaling pathways [5]. Exposure to UV radiation can lead to mitochondrial dysfunction, resulting in a decrease in ATP production, and disruption of cellular calcium channel regulation [6, 7]. This impacts mitochondrial membrane potential and permeability, eventually leading to cell death or apoptosis. Traditional sunscreens primarily provide a barrier against UV rays but their effects are often short-term and insufficient for targeting the underlying mitochondrial damage caused by UV exposure.

Despite the widespread use of sunscreens, studies have shown that most formulations do not effectively filter or reflect infrared (IR) radiation, leaving skin vulnerable to oxidative stress [8, 9]. Consequently, even with the application of sun protection products, prolonged exposure to intense sunlight can result in cumulative skin damage. While sunscreens with a high sun protection factor (SPF) can mitigate some damage from ultraviolet B (UVB) and ultraviolet A

(UVA) rays, they do not prevent the accumulation of damage caused by IR radiation [10, 11]. Both UV and IR rays can induce collagen degradation through different mechanisms, exacerbating the aging process [12, 13].

In recent years, natural extracts have gained attention for their potential to enhance skin health and protect against environmental stressors. Among these, sunflower sprout extract (Helianthus Annuus Sprout Extract, or HAS extract) has emerged as a promising candidate for various applications [14, 15]. However, its role in sunscreen formulations remains largely unexplored. Investigating whether HAS Extract can enhance the SPF of sunscreen formulations against specific or all wavelengths of UV light while also stimulating ATP production and modulating ROS levels, could lead to the development of more effective sunscreen formulations. Additionally, its potential to promote the survival of epidermal cells and provide protective benefits for cellular mitochondria would be crucial for understanding the full capabilities of HAS extract in skincare applications.

This study aims to innovate sunscreen formulations by integrating HAS extract, providing a dual-action approach: effective UV protection alongside mitochondrial protection. The developed sunscreen lotion is designed to absorb UV radiation across the 240-400 nm spectrum, effectively shielding the skin from harmful rays while promoting mitochondrial ATP synthesis in compromised cells. Unlike conventional formulations, this innovative product offers enhanced protection against medium and long UV wavelengths, addressing a significant gap in current sun care solutions. By exploring the application of HAS extract as a promising ingredient for mitochondrial protection in sunscreen formulations, our study aims to illuminate pathways for future developments in sun protection technology.

2. Materials and Methods

2.1 Materials

The experimental materials and instruments used in this study are as follows:

Materials: SkinEthic™ RHE model and culture medium, Human interleukin 1 Beta (IL-1β) ELISA kit (JONLNBIO), Reactive oxygen species (ROS) Assay kit (Beyotime Biotechnology), Mitochondrial membrane potential assay kit with 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolocarbo-cyanine iodide (JC-1) (Beyotime Biotechnology), ATP assay kit (Beyotime Biotechnology), Tissue mitochondria isolation Kit (Beyotime Biotechnology), and Sodium hydroxide (NaOH; Sangon).

Instruments: SpectraMax M Series Multi-Mode Microplate Reader (Molecular Devices, LLC), Ultraviolet-visible (UV-VIS) spectrophotometer (Hach® DR6000™), UV lamp (SANKYO DENKI, 15w，280nm), and TissueLyser II (Qiagen).2.2 Methods.

The blank cream and sunscreen formulation used in this study is currently under patent application; therefore, the detailed formulation cannot be disclosed. In brief, the sunscreen control used the same blank cream (vehicle) as the base, incorporating sunscreen actives but

excluding HAS extract. The SSE sunscreen formulation contained 2% sunflower sprout (HAS) extract.

Vehicle = blank cream
Sunscreen control = blank cream + sunscreen active (extruding HAS extract)
SSE Sunscreen = blank cream + sunscreen active (with 2% HAS extract)

2.2 Methods

2.2.1 Reactive oxygen species (ROS) measurement in UV-Damaged Cells

Reconstructed human epidermis (RHE) models were utilized to assess reactive oxygen species (ROS) levels in UV-damaged cells following various treatments. The RHE models were treated with 5 mg of either blank cream (vehicle), 2% sunflower sprout (HAS) extract-containing sunscreen (SSE sunscreen), or sunscreen control (without HAS) before being exposed to UV light (280nm) for 12 hours. The RHE model without sunscreen served as the UV damage control group (UV-NC group), while the RHE model maintained under normal culture conditions acted as the blank control group (NC group).
After treatments, the RHE models were collected and washed three times with 1X PBS, followed by incubation with 5 μM DCFH-DA-A for 45 min at 37˚C in the dark. The models were then washed three times with 1X PB, and cellular fluorescence was measured (Excitation: 488 nm; Emission: 525 nm). Data are presented as mean ± S.D. (n = 2), with significance indicated at **$p < 0.01$.

2.2.2 Mitochondrial adenosine 5'-triphosphate (ATP) production capacity
Intracellular ATP concentrations were assessed using an ATP assay kit n UV-damaged RHE models following various treatments. ATP levels were quantified by comparing the samples to a standard curve generated with known ATP concentrations. The data are presented as mean ± S.D. (n = 3), * $p < 0.05$, *** $p < 0.001$.

2.2.3 Mitochondrial membrane potential assay kit with JC-1
The Mitochondrial Membrane Potential Assay Kit with JC-1 was employed to assess changes in membrane potential ($\Delta\Psi m$) in UV-damaged RHE models following various treatments. After JC-1 staining at 37°C for 10 minutes, the RHE models were washed three times with JC-1 staining buffer and analyzed using a UV-VIS spectrophotometer.
In healthy mitochondria, JC-1 aggregates to form polymers in the mitochondrial matrix, emitting intense red fluorescence (Excitation: 525 nm; Emission: 590 nm). In unhealthy mitochondria, JC-1 remains in its monomeric form in the cytoplasm due to a decline or loss of mitochondrial membrane potential, resulting in green fluorescence (Excitation: 490 nm; Emission: 530 nm). Therefore, the ratio of red to green fluorescence reflects changes in mitochondrial membrane potential.
Data are presented as mean ± S.D. (n = 2), with significance indicated at $p < 0.01$ and $p < 0.001$.

2.2.4 Interleukin-1β (IL-1β) expression in UV-damaged cells

To determine the role of Interleukin-1 beta (IL-1β), the expression of IL-1β was assessed following various treatments using the Human IL-1β ELISA kit. Data are presented as mean ± SD (n = 2), with significance levels indicated as **p < 0.01 and ***p < 0.001.

2.2.5 UV-visible absorbance measurement

5 mg of the vehicle group, SSE sunscreen, and sunscreen control group were dissolved in 1 mL of NaOH. The UV absorbance of the resulting solutions was then measured using an ultraviolet-visible (UV-Vis) spectrophotometer.

3. RESULTS

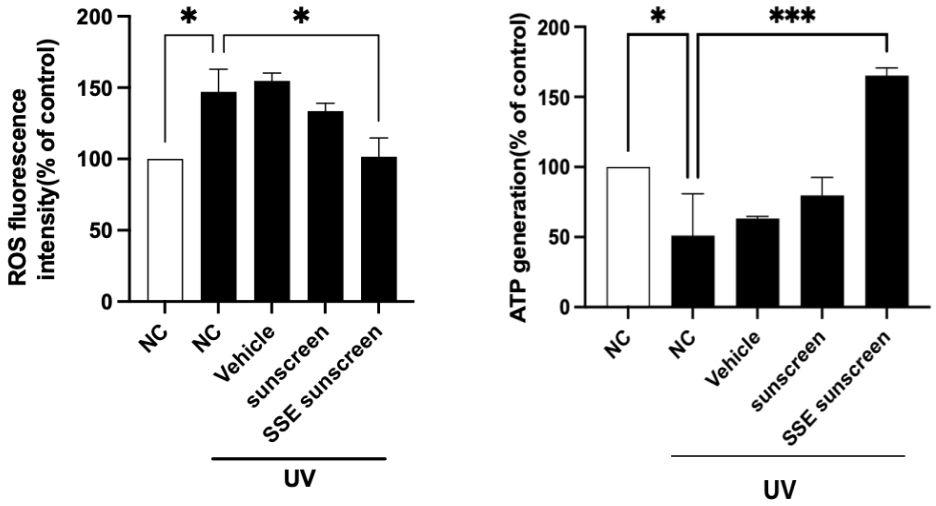

Figure 1. (a) ROS fluorescence intensity in UV-damaged cells after various treatment. (b) Mitochondrial ATP generation after treatments.

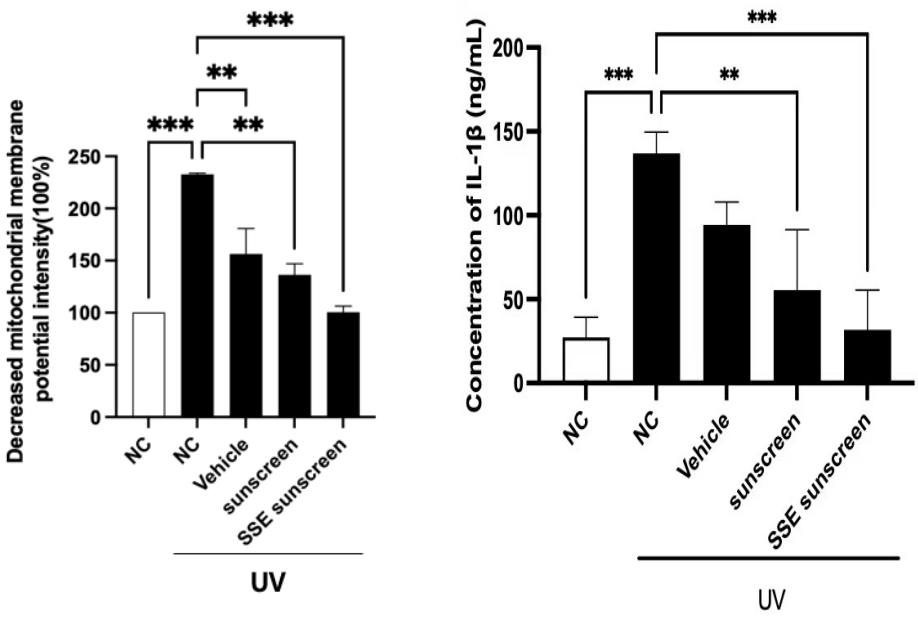

Figure 2. (a) Decrease in mitochondrial membrane potential after various treatment. (b) Concentration of IL-1β in UV-damaged cells after treatments.

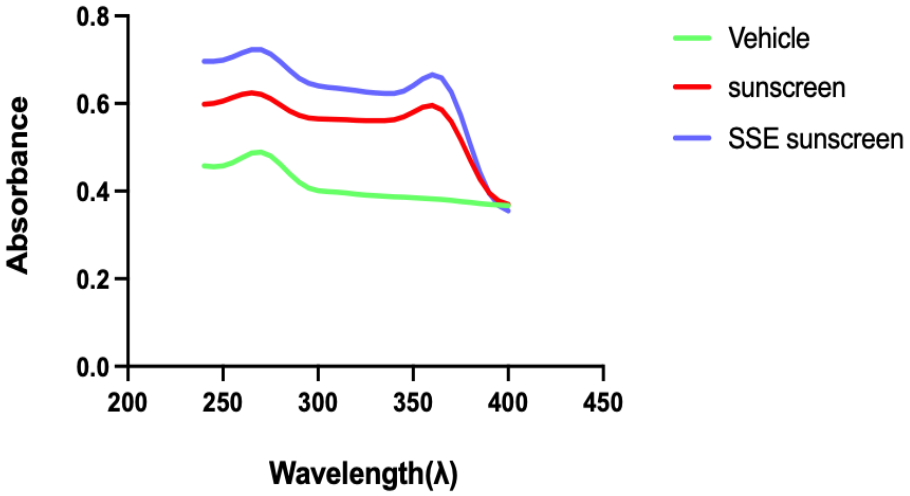

Figure 3. UV absorbance curves

4. DISCUSSION

4.1 Reactive oxygen species (ROS) measurement in UV-Damaged Cells

SkinEthic™ RHE model was used to evaluate the change in ROS levels after different treatments. These models closely mimic human skin so it can be used to assess the protective effects of the sunflower sprout extract and the potential efficacy of the sunscreen formulations. As shown in Figure 1a, UV exposure induced significant oxidative stress, evidenced by an increase in ROS levels. The SSE sunscreen significantly inhibited ROS levels (101.62%), bringing them back to baseline levels comparable to those of the cells under normal culture conditions (100%). In contrast, even with application of sunscreen control (133.52%) , ROS intensity remained over 33.52% higher in UV-damaged cells compared to the RHE model under normal culture conditions. While both the SSE sunscreen and the sunscreen control demonstrated reductions in ROS, SSE sunscreen exhibited a much greater inhibition, underscoring the effectiveness of HAS extract as a promising candidate for sun protection. Additionally, ROS generation exceeded 47.17% in UV-damaged cells when no protective treatment was applied, including the vehicle cream without sunscreen ingredients, compared to the normal culture condition.

Mitochondria are crucial organelles responsible for generating ATP, the primary energy source for cellular functions. Elevated ROS levels can adversely affect mitochondrial performance, leading to a decline in ATP production. This reduction in energy availability can have significant implications for overall cellular and tissue function, potentially contributing to cellular damage and dysfunction.

4.2 Mitochondrial ATP production capacity

Figure 1b illustrates that UV exposure significantly reduces ATP production, indicating a decline in the energy production capacity of damaged mitochondria. The results show that ATP

levels were markedly decreased following UV exposure. Both the sunscreen and vehicle cream treatments provided some degree of protection (80.96% and 63.62% respectively); however, these effects were not statistically significant compared to the NC group.

In contrast, the SSE sunscreen demonstrated exceptional efficacy, maintaining ATP production at levels comparable to the NC group (100%) and even enhancing ATP levels to 188.31% in UV-exposed cells. This finding suggests that SSE sunscreen not only protects mitochondrial health under UV stress but also effectively enhances ATP generation.

Together with the results obtained from the ROS assay, these findings illustrate the critical relationship between UV exposure and mitochondrial function, as evidenced by elevated ROS levels and decreased cellular energy production. The significant reduction in ATP levels in UV-damaged cells highlights the detrimental impact of oxidative stress on mitochondrial health. Conversely, treatment with SSE sunscreen not only enhanced ATP production but also reduced ROS levels, suggesting its potential as an effective tool in skincare.

4.3 Mitochondrial membrane potential

A decrease in mitochondrial membrane potential is an early indicator of apoptosis. Once the mitochondrial membrane potential drops, cells may enter an irreversible apoptotic process. Therefore, inhibiting the decline of mitochondrial membrane potential is crucial for preventing apoptosis. The mitochondrial membrane potential serves as a key indicator of the permeability of the mitochondrial inner membrane.

Figure 2a demonstrates that SSE sunscreen significantly inhibited the decrease in mitochondrial membrane potential in UV-damaged RHE models, compared to the vehicle and standard sunscreen controls. The levels observed with SSE sunscreen were 100.52%, approximately equal to those of the non-UV-exposed group（100%）. In contrast, the increase in mitochondrial membrane potential in the NC group after UV exposure was 232.59%, more than twofold, while the vehicle and sunscreen groups exhibited increases of over 36.37%. Consequently, SSE sunscreen shows considerable potential in protecting mitochondrial membrane potential from UV-induced damage, suggesting it may inhibit the onset of apoptosis and promote cellular health in skin cells. Further exploration of the mechanisms underlying its protective effects would be valuable.

4.4 Interleukin-1β (IL-1β) expression in UV-damaged cells

IL-1β is a crucial pro-inflammatory cytokine that is influenced by UV exposure and mitochondrial dysfunction. Elevated levels of IL-1β are often associated with cellular stress and apoptosis, indicating an inflammatory response to UV-induced damage. Both inflammation and mitochondrial dysfunction can lead to cell damage.

Figure 2b illustrates the impact of UV exposure on skin, showing that UV exposure significantly increases IL-1β expression. The concentration of IL-1β in the NC group after UV exposure increased dramatically to 136.96 ng/ml, indicating a substantial inflammatory response. The

vehicle-treated cells also exhibited elevated IL-1β levels (94.28 ng/ml), while the group treated with standard sunscreen showed a concentration of 100 ng/ml.
In contrast, the SSE sunscreen group markedly reduced IL-1β levels to 31.82 ng/ml, nearly bringing to the baseline levels as observed in the non-UV-exposed group (27.17 ng/ml). This suggests that SSE sunscreen effectively mitigates UV-induced inflammation, highlighting its protective role against skin damage.
The findings indicate a potential correlation between the decline in mitochondrial membrane potential and increased IL-1β production in UV-damaged cells. By protecting mitochondrial function and reducing oxidative stress, SSE sunscreen may help mitigate the inflammatory response mediated by IL-1β. Understanding the relationship between IL-1β levels and mitochondrial health could provide insights into the protective mechanisms of SSE sunscreen against UV-induced skin damage and inflammation. Further investigation into this relationship could enhance our understanding of how to effectively manage skin inflammation and maintain cellular integrity.

4.5 UV-visible absorbance measurement
As illustrated in Figure 3, the SSE sunscreen treatment group exhibited the most significant absorption of medium and long-wavelength ultraviolet rays, particularly in the range of 240-400 nm, compared to both the vehicle group and the standard sunscreen group. Additionally, SSE sunscreen showed higher absorbance than the sunscreen group within this wavelength range. Consequently, SSE sunscreen may provide better protection for the dermis, reducing damage to elastic and collagen fibers caused by ultraviolet rays and helping prevent skin issues such as tanning and sunburn.

5. Conclusion

Our study developed a sunscreen lotion incorporating Helianthus Annuus Sprout (HAS) Extract, which effectively absorbs UV radiation in the 240-400 nm range and promotes mitochondrial ATP production in damaged skin. This innovative formulation has been submitted for patent application, underscoring its novelty and potential for commercial development.
The study comprehensively assessed the protective effects of SSE sunscreen through various assays, including ROS, ATP, JC-1, and IL-1β expression, alongside UV absorbance measurements. These findings illuminate the potential of HAS Extract as an effective ingredient against mitochondrial dysfunction. Further exploration of the underlying mechanisms of these protective effects, as well as an investigation into the long-term benefits across different skin types and conditions, will be crucial for the development of innovative and effective sunscreen products.

REFERENCES

[1] Panich, U., Sittithumcharee, G., Rathviboon, N., & Jirawatnotai, S. (2016). Ultraviolet radiation-induced skin aging: the role of DNA damage and oxidative stress in epidermal stem cell damage mediated skin aging. *Stem cells international*, *2016*(1), 7370642.

[2] Salminen, A., Kaarniranta, K., & Kauppinen, A. (2022). Photoaging: UV radiation-induced inflammation and immunosuppression accelerate the aging process in the skin. *Inflammation Research*, *71*(7), 817-831.
[3] Burns, E. M., Ahmed, H., Isedeh, P. N., Kohli, I., Van Der Pol, W., Shaheen, A., ... & Yusuf, N. (2019). Ultraviolet radiation, both UVA and UVB, influences the composition of the skin microbiome. *Experimental dermatology*, *28*(2), 136-141.
[4] Kulms, D., & Schwarz, T. (2002). Molecular mechanisms involved in UV-induced apoptotic cell death. *Skin Pharmacology and Physiology*, *15*(5), 342-347.
[5] Brand, M. D., & Nicholls, D. G. (2011). Assessing mitochondrial dysfunction in cells. *Biochemical Journal*, *435*(2), 297-312.
[6] Paz, M. L., González Maglio, D. H., Weill, F. S., Bustamante, J., & Leoni, J. (2008). Mitochondrial dysfunction and cellular stress progression after ultraviolet B irradiation in human keratinocytes. *Photodermatology, photoimmunology & photomedicine*, *24*(3), 115-122.
[7] Birch-Machin, M. A., & Swalwell, H. (2010). How mitochondria record the effects of UV exposure and oxidative stress using human skin as a model tissue. *Mutagenesis*, *25*(2), 101-107.
[8] Vilela, F. M., Oliveira, F. M., Vicentini, F. T., Casagrande, R., Verri Jr, W. A., Cunha, T. M., & Fonseca, M. J. (2016). Commercial sunscreen formulations: UVB irradiation stability and effect on UVB irradiation-induced skin oxidative stress and inflammation. *Journal of Photochemistry and Photobiology B: Biology*, *163*, 413-420.
[9] Diffey, B., & Cadars, B. (2016). An appraisal of the need for infrared radiation protection in sunscreens. *Photochemical & Photobiological Sciences*, *15*(3), 361-364.
[10] Dupont, E., Gomez, J., & Bilodeau, D. (2013). Beyond UV radiation: a skin under challenge. *International journal of cosmetic science*, *35*(3), 224-232.
[11] Schroeder, P., Calles, C., Benesova, T., Macaluso, F., & Krutmann, J. (2010). Photoprotection beyond ultraviolet radiation–effective sun protection has to include protection against infrared A radiation-induced skin damage. *Skin pharmacology and physiology*, *23*(1), 15-17.
[12] Kamińska, A., & Sionkowska, A. (1996). Effect of UV radiation on the infrared spectra of collagen. *Polymer Degradation and Stability*, *51*(1), 19-26.
[13] Metreveli, N. O., Jariashvili, K. K., Namicheishvili, L. O., Svintradze, D. V., Chikvaidze, E. N., Sionkowska, A., & Skopinska, J. (2010). UV–vis and FT-IR spectra of ultraviolet irradiated collagen in the presence of antioxidant ascorbic acid. *Ecotoxicology and Environmental Safety*, *73*(3), 448-455.
[14] Barua, S., Jiang, L. I., Kononov, T., & Zahr, A. S. (2022). A case study investigating the short-term efficacy and tolerability of a daily serum composed from a unique sunflower sprout extract. *Journal of Cosmetic Dermatology*, *21*(10), 4410-4421.
[15] Becker, L. C., Boyer, I. J., Bergfeld, W. F., Belsito, D. V., Hill, R. A., Klaassen, C. D., ... & Heldreth, B. (2023). Safety Assessment of Helianthus annuus (Sunflower)-Derived Ingredients as Used in Cosmetics. *International Journal of Toxicology*, *42*(1_suppl), 93S-116S.