Boosting NAD⁺ levels for skin longevity

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ABSTRACT

The cosmetics industry is increasingly focusing on longevity, with a growing demand for maintaining a youthful appearance across all age groups. This article investigates the potential of sunflower sprout active, a product derived from organically grown sunflower sprouts, for promoting skin rejuvenation. *In vitro* and *ex vivo* studies have shown that sunflower sprouts can combat ageing processes by elevating NAD⁺ levels, enhancing DNA repair, improving mitochondrial function, and increasing collagen density. A subsequent clinical study revealed enhanced skin smoothness and facial lifting potential after application of 2% sunflower sprout active. Furthermore, the treatment noticeably reduced ageing signs, such as wrinkles and nasolabial folds, contributing to a youthful skin appearance. These findings suggest that sunflower sprout active can boost endogenous NAD⁺ levels, counteract fundamental ageing processes and optimize cellular processes. Overall, this research highlights the potential of sunflower sprout active in meeting the cosmetics industry's demand for youthful-looking skin, offering a holistic solution for skin rejuvenation.

The beauty industry is increasingly emphasizing the trending concept of longevity to meet the everlasting demand for vital, youthful skin and skin rejuvenation. This emphasis aligns with the historical pursuit for prolonged lifespan, which has driven advances in research related to ageing.

In 2013, the classical 'hallmarks of ageing' were introduced to define the fundamental biological processes underlying ageing.[1] These mechanisms are universal in all organisms experiencing biological ageing and result in impaired function, the gradual loss of physiological integrity, and ultimately, death.

The classical hallmarks of ageing from 2013 include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, cellular senescence, mitochondrial dysfunction, stem cell exhaustion, altered intercellular communication, and deregulated nutrient sensing.

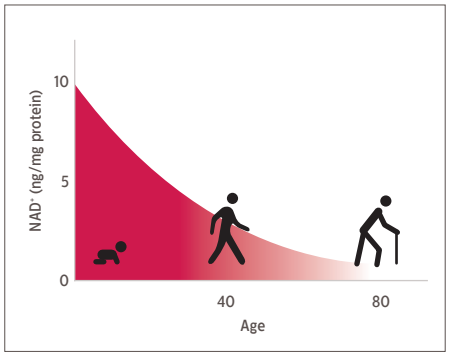

Figure 1: NAD⁺ declines with age

As our understanding of the biological mechanisms underlying skin ageing deepens, the concept of skin longevity is likely to become more central in skincare and skin rejuvenation, potentially leading to more targeted and effective treatments.[2]

The longevity metabolite: NAD⁺

Growing evidence suggests that the metabolite nicotinamide adenine dinucleotide (NAD⁺) significantly impacts all hallmarks of ageing.[3] In recent years, NAD⁺ has gained substantial attention, particularly in the dietary supplement industry, as studies have shown a robust correlation between declining NAD⁺ levels and accelerated ageing (Figure 1).[4]

NAD⁺ is a critical coenzyme in energy metabolism, particularly as it is essential for ATP production. In addition, NAD⁺ acts as a cofactor for hundreds of enzymes involved in various cellular processes, which are considerably relevant to all cell types, including skin cells.

In skin, inadequate NAD⁺ levels have been found to contribute to UV sensitivity, impaired DNA damage response, increased genomic instability, and the promotion of cellular senescence, so-called 'zombie cells', which in turn can lead to accelerated skin ageing.[5] However, incorporating NAD⁺ into cosmetics has presented challenges due to its instability in formulations and limited penetration into skin cells.

Superfood: organic sunflower sprouts

Sunflowers (*Helianthus annuus*), being heliotropic plants, exhibit the unique trait of following the sun's path across the sky. They symbolize faith, consistency, strength, warmth, and nourishment – qualities inherently linked to the sun.

To produce the sunflower sprout active, sunflower sprouts that are grown indoors in a controlled process, without the use of soil, are used. This method of indoor farming for cultivating organic sunflower sprouts offers numerous benefits. It provides a consistent supply of plant material, unaffected by seasonal changes, soil conditions, and market demand.

Moreover, it ensures plant material that is entirely devoid of environmental pollutants and pesticides. It also significantly reduces the water and farmland requirements compared to conventional farming methods.

Sprouts are known as superfoods, due to their high concentration of nutrients. During

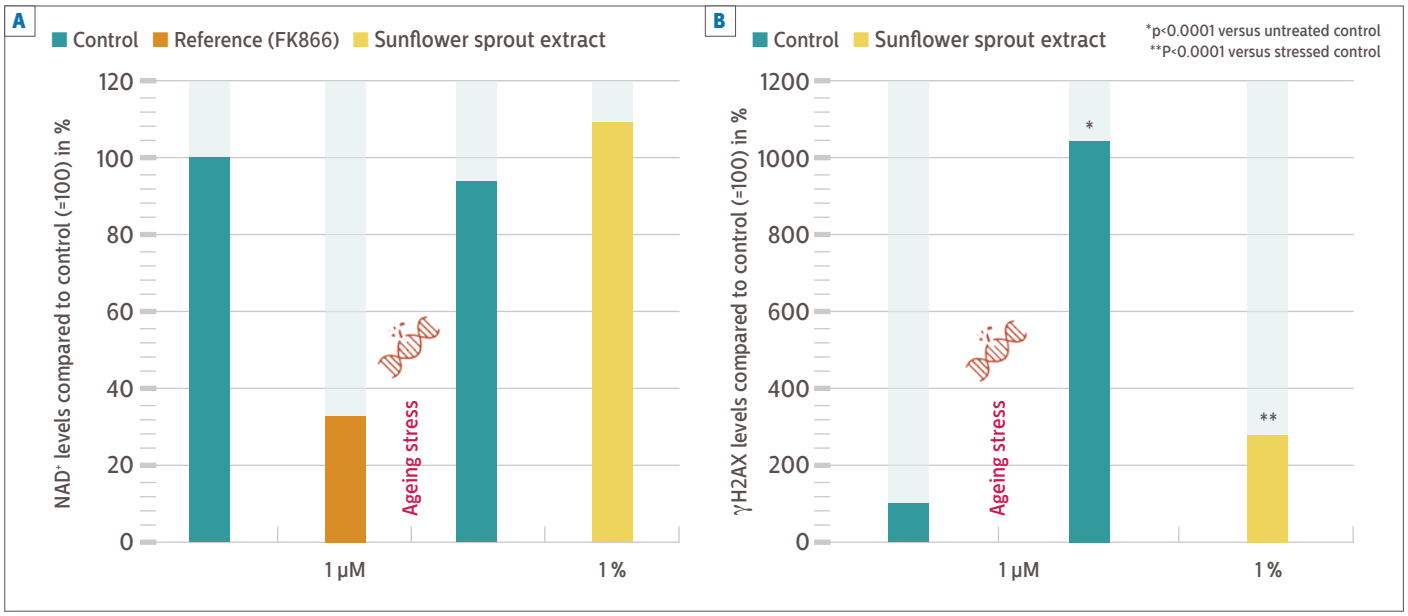

Figure 2: *In vitro* ageing model: Sunflower sprouts boost NAD⁺ levels and enhance DNA repair

the germination process, seeds undergo a transformation, leading to the conversion, and breakdown of various nutrients.[6] This process significantly enhances the nutritional value of the sprouts, turning them into a potent source of beneficial compounds.

Methods

In vitro ageing model

Keratinocytes were seeded in 24-well plates, either directly or on glass coverslips, and incubated for 24 hours. The cells were then pretreated with 1% sunflower sprout extract for an additional 24 hours. Ageing stress was induced with BrdU, and cells were further incubated for either 24 or 48 hours, depending on the subsequent analysis.

For the analysis of NAD⁺ levels, cells treated with BrdU in the absence or presence of 1% sunflower sprout extract for 24 hours were used. The compound FK866 was included as a control. FK866 is an inhibitor of the enzyme NAMPT, known to be the rate-limiting enzyme for replenishing NAD⁺ levels.

NAD⁺ levels were assessed in cell lysates using the NAD/NADHGlo™ assay in accordance with the manufacturer's protocol. NAD⁺ levels were recorded using the GloMax® Discover Microplate Reader.

For the analysis of DNA damage, cells grown on glass coverslips and treated with BrdU for 48 hours in the presence or absence of 1% sunflower sprout extract were used. Cells were fixed, and immunofluorescence staining was performed with a Phospho-Histone H2A.X (Ser139) antibody. Images were captured with a 20x objective, and a quantitative evaluation of the staining was conducted.

Rejuvenation study on skin explants

The skin explant, sourced from a 35-year-old Caucasian female donor, was cultured at 37°C with 5% CO_2. The culture medium was refreshed every 24 hours. To explore potential skin rejuvenation, the skin explants were initially exposed to 6 J/cm² UV-A radiation.

Subsequently, the skin explants were treated topically either with a placebo cream or a cream containing 2% sunflower sprout active. After applying the cream, the skin explants were incubated for 24 hours and then harvested for further evaluation.

To evaluate mitochondrial function and protein recycling in the skin explants, carbonylation of mitochondrial proteins as a marker of mitochondrial protein damage was analyzed. For this, the mitochondrial proteins were first extracted and then analyzed by immunoblotting using a specific fluorescent probe for detection.

Additionally, the collagen density of the skin explants was examined after the treatment. Sections of the skin explants were assessed using XPolar® technology for imaging. The collagen density analysis was conducted on the reticular dermis, the deeper layer of the dermis. This layer is characterized by its densely arranged collagen fibres, embedded within a supportive matrix, which is crucial for the maintenance of skin structure.

Clinical rejuvenation study

To evaluate the clinical efficacy of sunflower sprout active, a double-blind, randomized, and placebo-controlled study was conducted. In this study, 22 volunteers aged between 52 and 65 years (average age: 60 years) applied either a cream containing 2% sunflower sprout active or a corresponding placebo cream on each side of their faces twice daily for 42 days.

Various skin ageing parameters were measured after 28 and 42 days of application. These parameters included skin smoothness (data not shown), the lifting of the jawline region, and the depth of crow's feet wrinkles.

For the lifting analysis, 3D images of the face were captured using the Visia®-CR after 42 days of treatment. The lifting effect was analyzed by measuring the length of three vertical lines between the eyes and the jawline region. The depth of crow's feet wrinkles was measured on days 0, 28, and 42 using Primos 3D analysis.

In order to evaluate whether the volunteers appeared younger, the average values of wrinkle depth were compared with a reference dataset comprising over 300 women aged between 30 and 65 years. This dataset includes the recorded age and wrinkle depth of the volunteers.

Results and discussion

In vitro ageing model

The skin is subjected to a wide array of stress factors. Both external and internal stressors, including chronological ageing, can cause DNA damage. However, the skin's capacity to repair DNA damage declines with age.

As a result, DNA damage becomes a significant contributor to skin ageing. In the repair process of damaged DNA, NAD⁺ serves as a crucial cofactor, which is consumed and consequently depleted.

Previous work has shown that sunflower sprout extract can increase the expression of the rate-limiting enzyme nicotinamide phosphoribosyltransferase (NAMPT) that is responsible for NAD⁺ synthesis and recycling.

To simulate skin ageing, an *in vitro* skin ageing model in human epidermal keratinocytes was established using DNA damage. In this model, ageing stress is induced both in the absence and presence of 1% sunflower sprout extract by incorporating a thymidine analog, 5-bromodeoxyuridine (BrdU), into DNA, which triggers DNA damage.

To demonstrate the dependency of NAD⁺ levels on the enzyme NAMPT, the cells were additionally treated with the NAMPT inhibitor FK866. The inhibition of NAMPT resulted in a significant reduction in NAD⁺ levels within the cells, highlighting the importance of this enzyme in NAD⁺ recycling and synthesis.

Ageing stress by BrdU treatment caused a depletion of NAD⁺ within the cells. However, co-treatment of the stressed cells with sunflower sprout extract elevated the NAD⁺ levels even beyond those of the control (Figure 2A). These results suggest that sunflower sprout extract enhances the longevity molecule NAD⁺ in skin cells, even under ageing conditions.

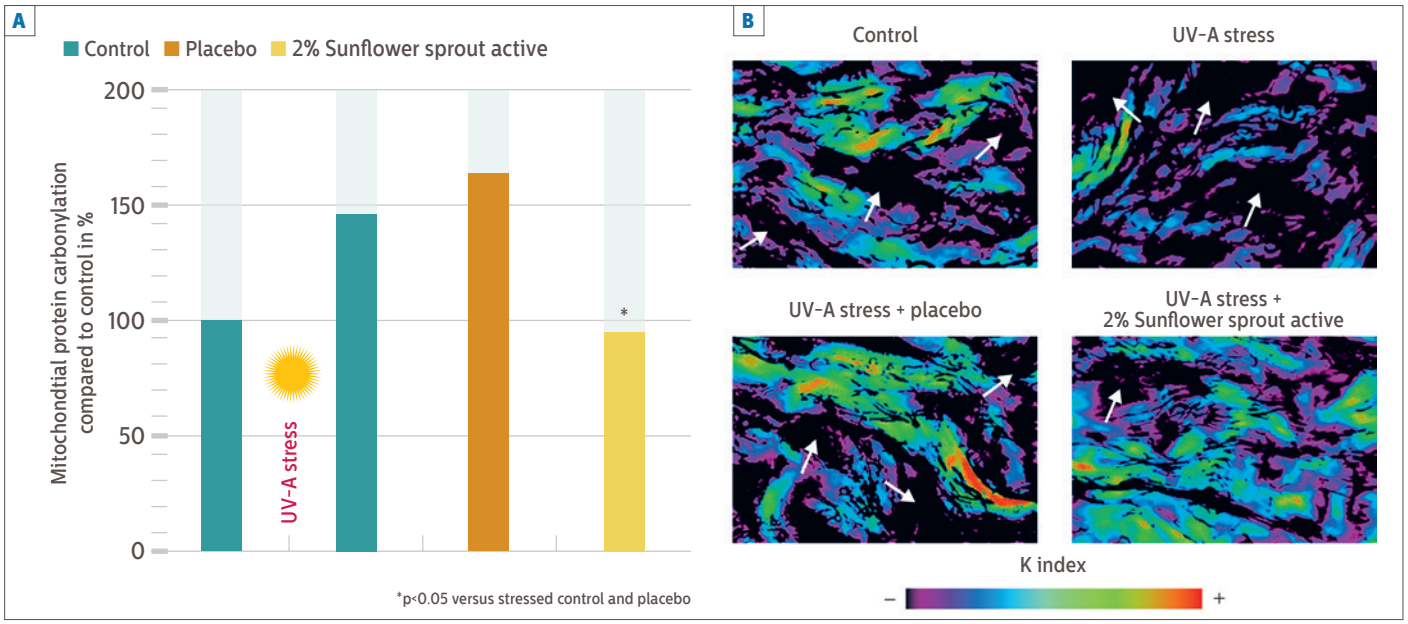

Figure 3: Rejuvenation study on skin explants: sunflower sprout active enhances mitochondrial function and collagen density

To evaluate the response to DNA damage the levels of the DNA damage response marker γH2AX were assessed via immunofluorescence analysis. Ageing stress induced a substantial increase in DNA damage. Co-treatment with sunflower sprout extract significantly reduced this damage compared to the stressed condition, indicating that sunflower sprout extract improves the DNA repair mechanism (Figure 2B).

Rejuvenation study on skin explants

Replenished NAD^+ levels are recognized for their potential to slow down and even reverse the ageing process. Consequently, the rejuvenation potential of sunflower sprouts was further investigated.

To assess this, skin explants were initially exposed to UV-A radiation to induce ageing stress and subsequently treated with either a cream containing 2% sunflower sprout active or a corresponding placebo.

To evaluate the effect of the sunflower sprout active on mitochondrial function and proteostasis, mitochondrial protein carbonylation, a marker of mitochondrial protein damage, was analyzed by immunoblotting. UV-A radiation induced an increase in mitochondrial protein carbonylation in the skin explants.

The application of sunflower sprout active significantly reduced the mitochondrial damage compared to the stressed condition as well as the placebo, returning it to levels similar to the ones of the unstressed control (Figure 3A).

The obtained rejuvenation results demonstrate that sunflower sprout active can support mitochondrial function as well as cellular metabolism, and thus potentially slow down the ageing process of skin.

Exposure to UV-A radiation plays a significant role in photoageing due to its ability to penetrate deeply into the dermis, leading to the degradation of collagen fibres.

Given these effects, the impact of sunflower sprout active on collagen density was investigated within the rejuvenation model. UV-A radiation was observed to significantly reduce the collagen density in skin explants.

However, the application of sunflower sprout active led to a substantial increase in collagen density when compared to the UV-A exposed control (Figure 3B). A higher K index corresponds to an increased density of collagen fibres.

The representative images of collagen density, obtained through XPolar® technology, illustrate the more uniform and denser distribution of collagen fibres after the use of 2% sunflower sprout active. This is evidenced by the more evenly distributed blue-green areas and a reduction in black, low-density zones, indicative of strengthened skin connective tissue.

These findings suggest that sunflower sprout active can effectively counteract

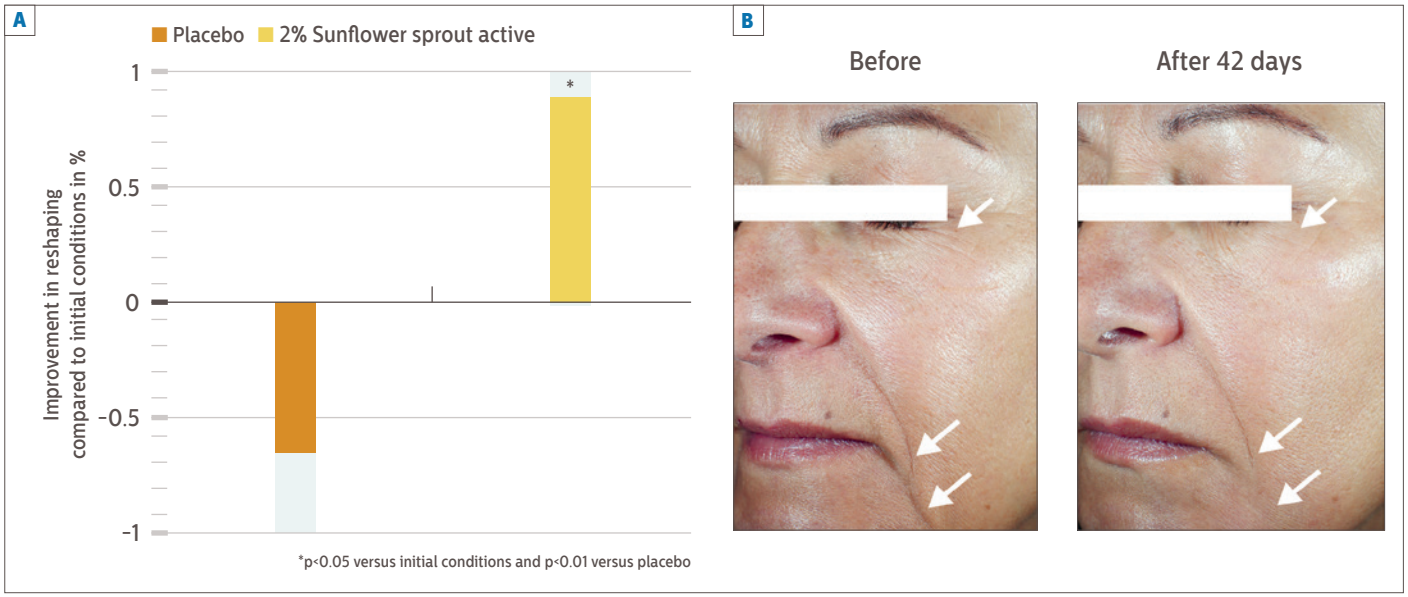

Figure 4: Clinical rejuvenation study: sunflower sprout active induces a lifting effect and visibly improves signs of skin ageing

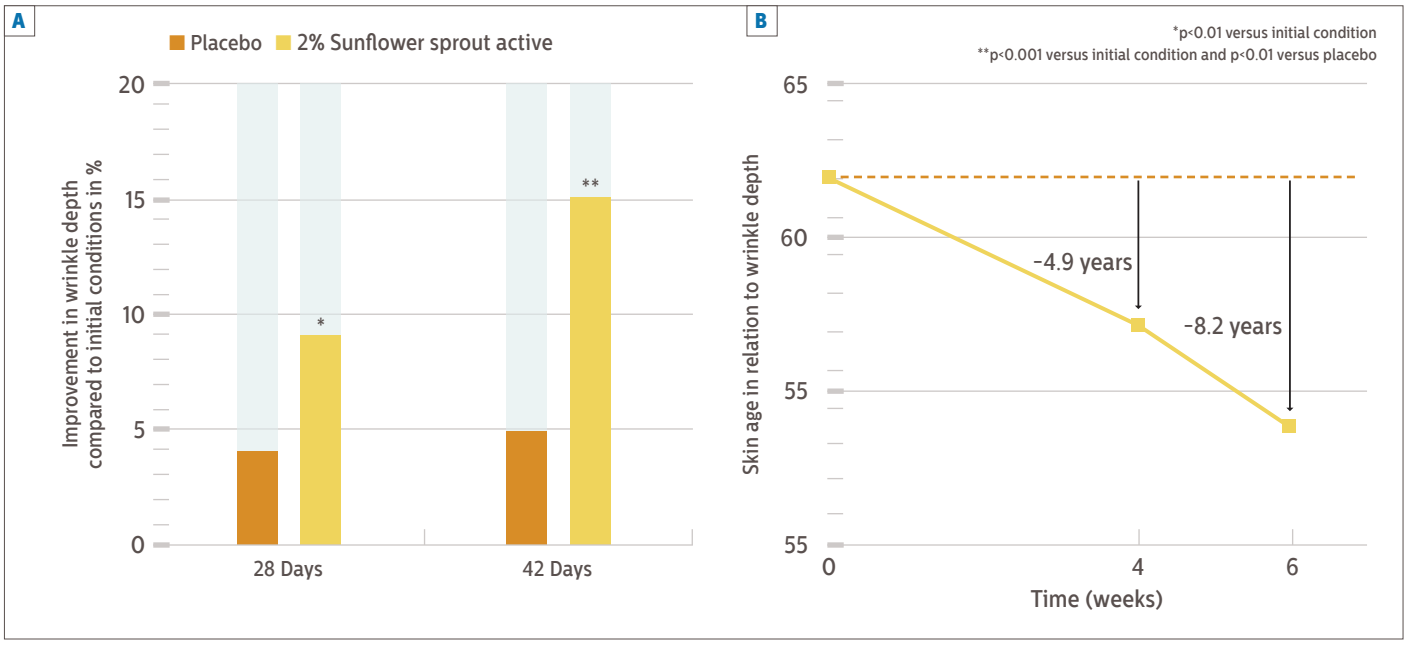

Figure 5: Clinical rejuvenation study: sunflower sprout active leads to wrinkle reduction and a more youthful skin appearance

the adverse effects of UV-A radiation on mitochondrial protein damage and collagen density, highlighting its potential to reverse the processes associated with ageing.

Clinical rejuvenation study

In vitro and *ex vivo* efficacy studies have demonstrated that sunflower sprouts can counteract ageing processes, potentially rejuvenating aged skin. Therefore, the clinical study aimed to investigate the impact of sunflower sprout active on skin rejuvenation.

The application of 2% sunflower sprout active significantly improved skin smoothness after 28 and 42 days compared to the initial condition (data not shown). In addition to skin smoothness, assessments were conducted to investigate the facial lifting potential of sunflower sprout active.

The smaller the distances between the reference points at the eye and jaw line region, the more pronounced the lifting effect. Treatment with 2% sunflower sprout active led to a lifting of the jawline region.

The length of the measured vertical lines was significantly reduced by more than 1.5% compared to the placebo (Figure 4A). Images of a volunteer captured with the Visia®-CR demonstrated a visible improvement in ageing signs.

The representative pictures showed a facial reshaping by reducing the crow's feet wrinkles, the nasolabial fold, and the marionette fold, highlighting that sunflower sprout active visibly and effectively rejuvenates the skin (Figure 4B).

The visual improvement in facial ageing signs was complemented by a quantitative analysis of the impact of sunflower sprout active on wrinkles. The application of sunflower sprout active resulted in a significant reduction in the depth of wrinkles compared to the initial condition, with reductions of 9.2% and 15.3% after 28 and 42 days, respectively. After 42 days, the improvement in wrinkle depth was also significant compared to the placebo (Figure 5A).

Furthermore, the comparison of the wrinkle depth measured in this study with a reference dataset revealed that sunflower sprout active made the skin appear on average 4.9 and 8.2 years younger after 28 and 42 days, respectively (Figure 5B).

Overall, the findings of the clinical study demonstrate that sunflower sprout active rejuvenates the skin and contributes to a more youthful-looking appearance.

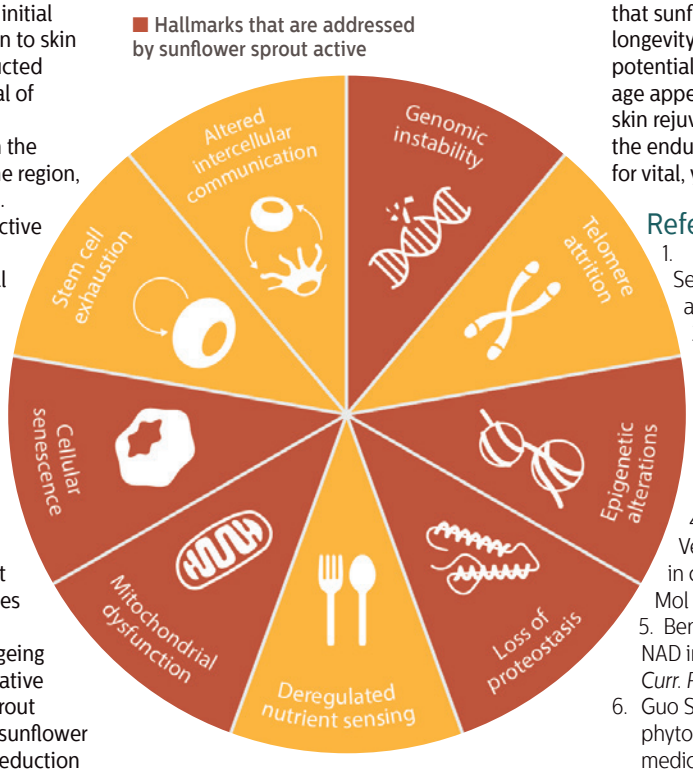

Figure 6: Sunflower sprout active tackles the hallmarks of ageing

Conclusion

The sunflower sprout active, derived from organically cultivated sunflower sprouts through sustainable indoor farming, has been shown to naturally enhance endogenous NAD^+ levels. The extract from the sunflower sprouts invigorates mitochondria, promotes DNA repair, and optimizes cellular processes.

The data from prior research, in conjunction with the findings from this study, have unveiled that sunflower sprout active targets at least five of the classical hallmarks of ageing, the very essence of age-related processes (Figure 6).

Moreover, clinical findings have revealed that sunflower sprout active promotes skin longevity by visibly rejuvenating the skin, potentially taking eight years off one's visual age appearance. With its holistic approach to skin rejuvenation, this active ingredient meets the enduring demand of the cosmetic industry for vital, youthful-looking skin. **PC**

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