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Mitochondrial Rejuvenation from Within: Plant Stem Cell Breakthroughs to Reverse Skin Aging and Restore Radiance

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1. Introduction

Skin aging is a process intricately linked to the decline in cellular functions, leading to observable changes in the skin's physical characteristics. At the cellular level, the mitochondrial aging theory is widely recognized as one of the primary contributors to the aging process [1]. Mitochondria, which serve as the principal generators of cellular energy, are also major sites of free radical production. Approximately 2–5% of the excess free radicals produced during energy generation can damage mitochondria. Additionally, mitochondrial dysfunction, induced by aging, further exacerbates free radical accumulation [2]. Research has demonstrated that mitochondrial membrane potential (MMP) serves as a critical indicator of mitochondrial function. MMP is positively correlated with free radicals, and an excess of free radicals can reduce MMP, ultimately leading to mitochondrial dysfunction [3]. Therefore, restoring normal MMP presents a key strategy for improving mitochondrial function.

Plant stem cells, which are undifferentiated cells derived from plant meristematic tissues, possess remarkable abilities for self-renewal and multi-directional differentiation. These properties have generated increasing research interest, particularly in the field of skincare. Plant stem cells offer significant benefits, including the promotion of skin cell regeneration and repair, antioxidant and anti-aging effects, and potential skin whitening properties.

The extraction and cultivation of plant stem cells represent one of their major technical challenges in their practical application. By employing advanced laboratory-based techniques, our previous research has established an effective method for plant stem cell extraction, termed the Phyto-To-Cell technique (PCT). This process not only minimizes environmental impact but also reduces the burden on water and soil resources, thereby contributing to sustainable development[4]. Through this innovative approach, single plant stem cells can be obtained, retaining the complete spectrum of plant cellular constituents. These include epigenetic molecules such as miRNA, exosomes, proteins, as well as secondary metabolites like polysaccharides. Scientific evidence has demonstrated that these components act synergistically to promote cell proliferation and differentiation, thereby counteracting the signs of aging and offering promising anti-aging skincare solutions [5,6].

Despite their potential, the phytochemical constituents of plant stem cells—such as saponins, alkaloids, and polyphenols—remain insufficiently studied. In particular, the synergistic mechanisms through which these compounds exert anti-aging effects are not yet well understood. Notably, investigating the role of multi-plant stem cell extracts in restoring mitochondrial function is of significant value, as many underlying mechanisms remain unknown. This study aims to explore the effects of plant stem cell combinations enriched with these phytochemical components on the enhancement of mitochondrial function, reversal of skin aging and restoration of skin radiance from within.

2. Materials and Methods

2.1 PCTs Complex Composition

As shown in Table 1, a Swiss-derived plant stem cell complex containing three components—Saponaria officinalis stem cells (Sas), Symphytum officinale stem cells (Sys), and Leontopodium alpinum stem cells (Les)—was extracted using the Phyto-To-Cell (PCT) method. This complex is referred to as PCT. A second complex named Suissnergy was designed, comprising Melissa officinalis stem cells (Mes), Helianthus annuus sprout extract (Hes), and epigallocatechin galloyl glucoside (Epg). The combined formulation of PCT and Suissnergy is referred to as PCTs.

Table 1. Chemical Composition of PCTs

Combination		Raw Material	Active Ingredients
PCTs	PCT	Sas	Saponins from Saponaria officinalis [7]
		Sys	Alkaloids and polyamines [8]
		Les	Leontopodic acid [9]
	Suissnergy	Mes	triterpenes (ursolic acid and oleanolic acid), phenolic compounds (rosmarinic acid, caffeic acid, and protocatechuic acid) and flavonoids (quercetin, rhamnocitrin, luteolin) [10]
		Hes	Chlorogenic acid derivatives (CQAs): Caffeic acid and its derivatives, such as mono-, di- and tri-caffeoyl esters of quinic acid [11]
		Epg	Epigallocatechin gallate

2.2.. Antioxidant Activity by DPPH Assay

A total of 0.005 g of DPPH (1,1-diphenyl-2-picrylhydrazyl) powder was dissolved in anhydrous ethanol, ultrasonicated in the dark, and then diluted to a final volume of 100 mL to prepare a 50.0 μg/mL DPPH solution. Samples were subsequently diluted with purified water. For the experimental group, 1 mL of the sample was mixed with 3 mL of DPPH solution; for the control group, 1 mL of the sample was mixed with 3 mL of anhydrous ethanol; and for the blank group, 1 mL of solvent was mixed with 3 mL of DPPH solution. After incubation in the dark for 30 minutes, 200 μL of each reaction mixture was transferred to a 96-well plate (n3). Absorbance was measured at 517 nm, and free radical scavenging rates were calculated.

2.3. Mitochondrial Membrane Potential (MMP)

HaCaT cells were seeded in 12-well plates at a density of 5×10^4 cells per well and incubated at 37°C with 5% CO_2 for 24 hours. Experimental groups included a normal control, a model control, and sample treatment groups. Following UVB irradiation at a dose of 100 mJ/cm², cells were washed with D-Hanks solution. The sample groups were treated with the test complexes, while control groups received complete medium. After 24-hour incubation, JC-1 staining was performed, and the red/green fluorescence intensity ratio was analyzed using ImageJ software to assess MMP.

2.4. COL3A1 Gene Expression

Normal human dermal fibroblast (NHDF) was seeded into 6-well plates at a density of 1.5×10^5 cells per well and incubated for 24 hours. The experimental groups included a normal control group and treatment groups with the sample of interest. After 72 hours of treatment, total RNA was extracted from the cells, reverse transcribed into complementary DNA (cDNA), and analyzed by quantitative real-time PCR (qPCR) to evaluate the expression of the COL3A1 gene. (COL3A1 primers: forward 5′-AAGTCAAGGAGAAAGTGGTCG-3′ and reverse 5′-CTCGTTCTCCATTCTTACCAGG-3′.GAPDH primers: forward 5′-GTCTCCTCTGACTTCAACAGCG-3′ and reverse 5′-ACCACCCTGTTGCTGTAGCCAA-3′.)

2.5. Clinical Trial

Thirteen female volunteers (aged 46–60 years, mean age 53 ± 5 years) with visible signs of dullness, decreased elasticity, wrinkles, and skin sagging were enrolled in a 28-day clinical trial. Participants applied Suissnergy and PCTs formulations to one side of the face twice daily. Skin condition was evaluated at baseline (Day 0) and after treatment (Day 28). Descriptive statistics, including mean, standard deviation, maximum, minimum, and median values, were calculated for all parameters. Statistical analyses were performed using SPSS software. Normality of the differences between time points was assessed for each group. If data followed a normal distribution ($P > 0.05$), a paired t-test was used; otherwise, the Wilcoxon signed-rank test was applied. All statistical tests were two-tailed, and a significance level of $\alpha = 0.05$ was adopted.

Table 2. Composition and Corresponding Concentrations

Experiment	Combination	Composition concentration % w/w					
		Sas	Sys	Les	Mes	Hes	Epg
DPPH	PCT	0.5%	0.5%	0.5%	/	/	/
	Suissnergy	/	/	/	0.01%	0.01%	0.002%
	PCTs	0.5%	0.5%	0.5%	0.01%	0.01%	0.002%
MMP	PCT	0.001%	0.001%	0.001%	/	/	/
	Suissnergy	/	/	/	0.01%	0.01%	0.002%
	PCTs	0.001%	0.001%	0.001%	0.01%	0.01%	0.002%
COLIII	PCT	0.001%	0.001%	0.001%	/	/	/
	Suissnergy	/	/	/	0.025%	0.5%	0.05%

Clinical trial	PCTs	0.001%	0.001%	0.001%	0.025%	0.5%	0.05%
	suissnergy™	/	/	/	1.5%	2.0%	0.02%
	PCTs	1%	1%	2%	1.5%	2.0%	0.02%

3. Results

3.1. Antioxidant Activity

The plant stem cell combinations demonstrated antioxidant potential, attributed to the presence of phenolic compounds, saponins, and alkaloids. As shown in Figure 1, the DPPH radical scavenging rates for PCT, Suissnergy, and PCTs were 23.31%, 50.43% and 67.87%, respectively, following the order: PCTs > Suissnergy > PCT. The combination of PCT and Suissnergy exhibited a synergistic effect, significantly enhancing the overall antioxidant activity.

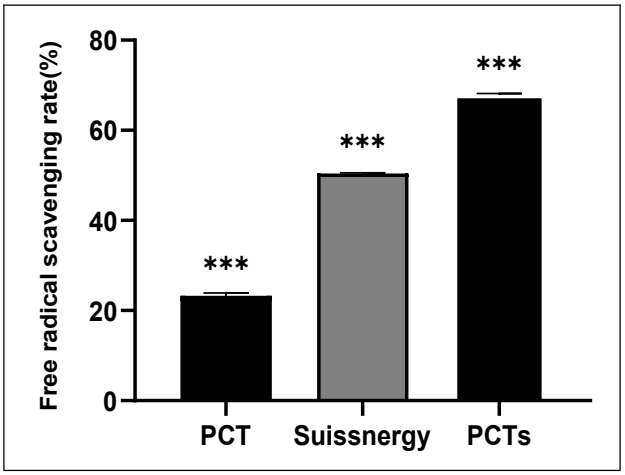


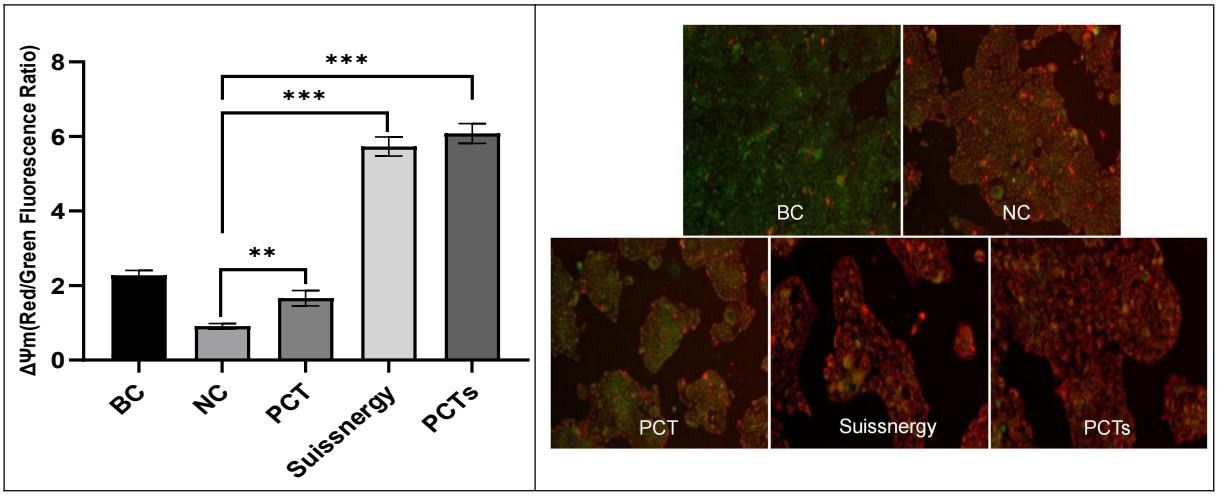
Figure 1. DPPH radical scavenging activity assay. Data are presented as the percentage of control based on three independent experiments, and expressed as mean ± standard deviation. Statistical significance was determined using Student's t-test: *p < 0.05, 0.001 ≤ **p < 0.01, and ***p < 0.001.

3.2. Mitochondrial Function Improvement

MMP serves as an important indicator of mitochondrial integrity and function. A reduction in MMP reflects mitochondrial dysfunction, decreased viability, and diminished cellular energy level [12]. Under normal conditions, JC - 1 accumulates in the mitochondrial matrix to form red-fluorescent aggregates; however, when mitochondria are damaged and MMP decreases, JC - 1 remains in monomeric form and emits green fluorescence. Therefore, the red-to-green fluorescence intensity ratio provides a quantitative measure of MMP, where a higher ratio corresponds to improved mitochondrial viability and energy production.

To assess the effects of plant stem cell extracts on mitochondrial function, HaCaT cells were treated with PCT, Suissnergy, and PCTs for 24 hours. Following treatment, JC-1 staining was performed and fluorescence intensity was measured. The red/green fluorescence ratio was used to evaluate changes in MMP. As shown in Figure 2a, compared to the model control group (b), the mitochondrial membrane potential increased by 98%, 532%, and 571% in the PCT, Suissnergy, and PCTs groups, respectively. The enhancement of MMP followed the

order: PCTs > Suissnergy > PCT, indicating that all three treatments significantly improved mitochondrial function, with the PCTs combination showing the most pronounced effect.



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

Figure 2. (a) The red/green fluorescence intensity ratio (the model group (BC), the negative control (NC), refers to the model group with UVB stimulation). (b) The fluorescence images. All data were expressed as a percentage of control from three independent experiments with the mean ± standard deviation and analyzed using Student's t-tests,*p < 0.05, 0.001 ≤ **p < 0.01, ***p < 0.001.

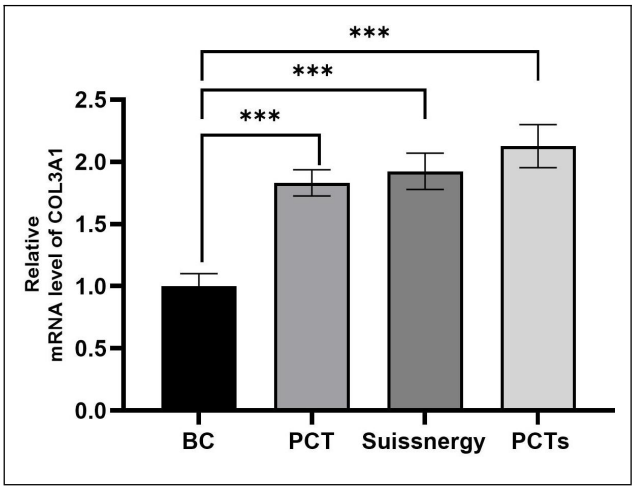


Figure 3. The plant stem cells have enhanced the relative mRNA level of COL3A1 compared to BC.All data were expressed as a percentage of control from three independent experiments with the mean ± standard deviation and analyzed using Student's t-tests, *p < 0.05, 0.001 ≤ **p < 0.01, ***p < 0.001.

3.3. COL3A1 Gene Expression

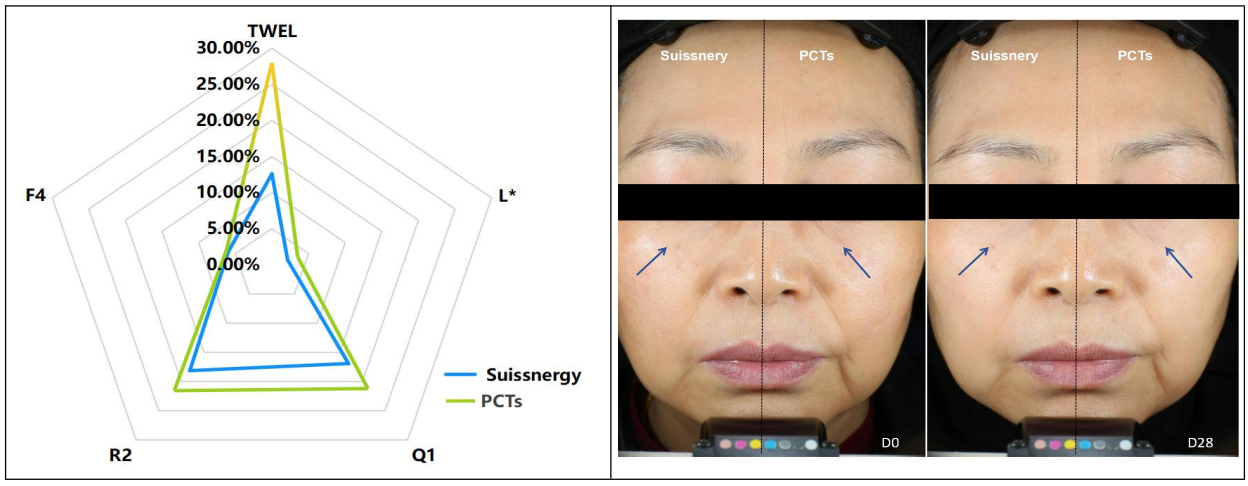
Collagen plays a crucial role in maintaining the skin's firmness and elasticity [13]. The synthesis of collagen in the skin is regulated by several genes, which are pivotal in the biosynthesis of collagen [14]. Among these, the COL3A1 gene is responsible for the production of type III collagen. Upregulating COL3A1 gene expression can enhance the synthesis of type III collagen [15].

To evaluate the effects of the three plant stem cell complex combinations on skin firmness and elasticity, we performed a COL3A1 gene expression analysis on PCT, Suissnergy and PCTs. The experimental results revealed that the expression levels of the COL3A1 gene for PCT, Suissnergy, and PCTs were 1.831, 1.924, and 2.126 respectively. All treatments significantly promoted COL3A1 expression compared to the blank control group ($P < 0.001$). These findings suggest that the three combinations can enhance skin firmness and elasticity by upregulating COL3A1 expression. Moreover, the superior effect observed in the PCTs group further demonstrates the synergistic interaction between PCT and Suissnergy.

3.4. Clinical trial

Two formulations, Suissnergy and PCTs, which demonstrated superior MMP enhancement and COL3A1 gene expression in vitro, were selected for human clinical evaluation. Thirteen female volunteers were assigned to apply formulations containing either Suissnergy or PCTs twice daily for a duration of 28 days. Skin parameters—including transepidermal water loss (TWEL), L* (skin brightness), R2 (skin elasticity), Q1 (smoothness), and F4 (firmness)—were measured using probe-based instruments, and improvements were observed across all dimensions (Figure 4a). Additionally, wrinkle analysis was conducted using Visio 4D in eight volunteers following the same application protocol.

As shown in Figure 4, both Suissnergy and PCTs significantly improved wrinkle appearance, with wrinkle area reduction rates of 7.91%* and 29.79%**, respectively (Figure 4b). These findings indicate that both formulations effectively address signs of skin aging, with PCTs exhibiting superior overall performance. Moreover, the enhanced efficacy of PCTs further supports the synergistic benefits of combining PCT and Suissnergy components.



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

Figure 4. Randomized half-face application of creams containing Suissnergy and PCTs for 28 days. (a) Compared to baseline (D0), significant improvements were observed in TWEL, L*,

Q1, and R2, as illustrated in the radar chart. (b) Wrinkle analysis using Visio 4D revealed a noticeable reduction in periorbital wrinkles in both treatment groups, with more pronounced improvement observed in the PCTs group.

4. Discussion

In our study, both plant stem cell composition PCT (composed of Sas, Sys) and Suissnergy (composed of Mes, Hes, and Epg) showed antioxidant potential in the DPPH assay, while their combination of PCT and Suissnergy, PCTs demonstrated a significantly enhanced effect. This synergy may stem from a more comprehensive blend of active ingredients, including saponins, alkaloids, and polyphenols.

MMP assays further confirmed the exceptional ability of these combinations—especially PCTs—to restore mitochondrial activity, resulting in a 571% increase in MMP ($P < 0.001$), far surpassing the effects observed in antioxidant tests. This indicates that, in addition to scavenging free radicals, the plant stem cell complex may directly enhance mitochondrial function, supporting the maintenance of cellular energy homeostasis.

Given the central role of mitochondria in skin aging, we further examined the impact on COL3A1 expression related to collagen synthesis. All treatments significantly upregulated this gene, supporting their efficacy in improving skin firmness and elasticity. Although the synergy in collagen expression was not as striking as in mitochondrial restoration, it nonetheless suggests a multi-pathway anti-aging mechanism. Additionally, preliminary data (not shown) suggest PCTs also promote cell proliferation and viability, pointing to further biological benefits worth exploring.

To translate these findings into real-world relevance, a 28-day clinical trial was conducted. Both Suissnergy and PCTs significantly improved key aging indicators including TWEL, L*, Q1, R2, and wrinkle area, with PCTs consistently outperforming Suissnergy. These results highlight the clinical potential of plant stem cell combinations to restore skin barrier function, improve elasticity and brightness, and reduce signs of aging. While mitochondrial enhancement appears to play a core role, the broader skin improvements suggest that other mechanisms are also involved, warranting deeper investigation in future studies.

Through systematic in vitro and clinical experiments, we confirmed that Suissnergy and PCTs exhibit strong anti-aging potential, likely through mechanisms closely associated with mitochondrial function, as illustrated in the schematic diagram in Figure 5 [16].

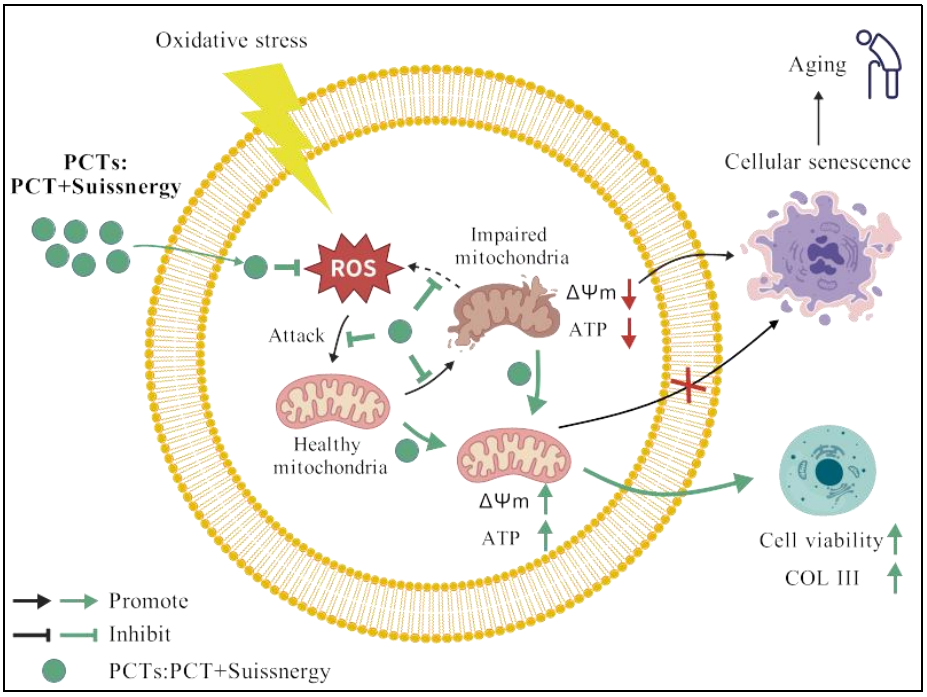


Figure 5. Mechanisms of Mitochondrial Function Boost by Plant Stem Cells.

5. Conclusion

In this study, through both in vitro and clinical experiments, we confirmed that the plant stem cell compositions Suissnergy (comprising Mes, Hes, and Epg) and PCTs (comprising Sas, Sys, Les, Mes, Hes, and Epg) exhibited strong anti-aging potential. Our findings demonstrate that these plant stem cell combinations possess potent antioxidant properties, significantly enhance mitochondrial membrane potential, boost cellular energy levels, and contribute to improved skin health and appearance. By targeting mitochondrial dysfunction, a fundamental driver of skin aging, this approach offers a promising strategy to restore youthful vitality and address the visible signs of aging at their root cause.

Furthermore, this study provides innovative insights into the synergistic use of different plant stem cell sources, laying a data foundation for elucidating their mechanisms of action. The in vitro and clinical evidence presented here highlights the potential of plant stem cells in the development of next-generation, mitochondria-targeted anti-aging formulations. Importantly, the use of plant stem cell-derived raw materials also aligns with the goals of sustainable development by reducing dependence on traditional agricultural resources and minimizing environmental impact. We anticipate that the broader application of plant stem cell technologies will unlock new possibilities for green, effective, and sustainable cosmetic solutions in future research.

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