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"Study on the extraction of highly active peptides from lotus flower proteins and evaluation of their anti-aging efficacy"

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1. Introduction

Skin aging has emerged as a paramount concern among consumers, with 72% of individuals aged 30-60 prioritizing anti-aging solutions in their skincare regimens. Through systematic analysis of aging populations, we propose a novel classification framework differentiating epidermal senescence ("tone aging") from dermal senescence ("texture aging"). Superficial manifestations of aging, termed tone aging, predominantly involve pigmentary disorders such as hyperpigmentation, uneven skin tone, and solar lentigines, driven by melanocyte hyperactivity and UV-induced oxidative stress. In contrast, texture aging, the focus of this study, encompasses deeper structural deterioration, including wrinkle formation, loss of elasticity, and gravitational sagging, which correlate with collagen depletion and extracellular matrix degradation.

Despite 60% of consumers identifying texture aging as their primary concern, current market offerings predominantly target surface-level corrections, neglecting the biomechanical underpinnings of dermal collapse. This work addresses this critical gap by introducing a lotus-derived nonapeptide that directly modulates fibroblast-mediated collagen synthesis, offering a mechanistically grounded solution to counteract multidimensional texture aging.

Contemporary interventions for texture aging predominantly rely on synthetic bioactive compounds, with 86% of FDA-approved dermal anti-aging agents classified as retinoids or engineered peptides. While retinols enhance collagen synthesis by 1.8-fold at 0.1% concentration, their clinical utility is hampered by phototoxicity (28% incidence in Fitzpatrick skin types IV–VI) and epidermal barrier disruption (transepidermal water loss increase $\geq 25\%$). Synthetic peptides, notably palmitoyl tripeptide-38, demonstrate moderate efficacy in fibroblast activation but suffer from conformational instability, with only 32% retaining native α -helix structures post-formulation. Furthermore, chemical synthesis routes introduce racemization artifacts (D-isomer contamination $> 18\%$), diminishing receptor-binding specificity and bioavailability. These limitations highlight an unmet demand for naturally derived, stereochemically precise alternatives that synergistically target collagen biosynthesis and matrix stabilization.

Lotus nine peptides emerge as a breakthrough anti-aging agent, uniquely addressing both collagen depletion and glycation—the dual drivers of texture aging. At 0.01% concentration,

lotus nine peptides stimulated Type I collagen secretion to 642.88 ng/mL ($p < 0.05$), outperforming acetyl hexapeptide-8 (335.46 ng/mL) and snake venom-like peptide (537.05 ng/mL) by 91.7% and 19.7%, respectively. Its efficacy extended to Type III (26.87 ng/mL, $p < 0.05$) and Type IV collagen (35.06 ng/mL, $p < 0.001$), demonstrating broad-spectrum matrix restoration. Concurrently, lotus nine peptides exhibited exceptional anti-glycation activity (IC₅₀: 0.334 mg/mL), rivaling pharmaceutical-grade aminoguanidine sulfate (IC₅₀: 0.308 mg/mL) and surpassing common agents like carnosine (IC₅₀: 3.2 m/mL) by 9.6-fold. This dual functionality eliminates the trade-off between collagen stimulation and glycation defense seen in retinoids and synthetic peptides. Dose synergy was evident at 0.005%, where collagen synthesis (1.43-fold COL17A1 upregulation, $p < 0.01$) coincided with 52% AGE suppression ($p < 0.05$). Derived from *Nelumbo nucifera* 'Xuanlian', a Tang Dynasty imperial tribute cultivar, lotus nine peptides achieve these effects at 1/10th the dosage of conventional actives, validated by ELISA, and qRT-PCR models. Its historical use in traditional skincare, combined with modern mechanistic validation, positions lotus nine peptides as a cost-effective, multifunctional solution to texture aging, bridging ethnobotanical wisdom with cutting-edge cosmeceutical science.

2. Materials and Methods

Human foreskin fibroblasts (HFF-1) were cultured in DMEM/10% FBS and treated with lotus nine peptides (0.003–0.01%), commercial peptides, or controls for 24 h. Type I/III/IV collagen

levels in supernatants were quantified via ELISA. Transdermal permeation was assessed using confocal Raman spectroscopy (785 nm laser, 72 µW, 0–120 µm depth) on human forearm skin. Anti-glycation activity was evaluated in a BSA-methylglyoxal model by measuring AGEs fluorescence (IC_{50} calculation). Proteomic profiling of lotus seed extracts involved tryptic digestion, LC-MS/MS (Q Exactive™ HF-X), and database searches (Nelumbo nucifera UniProt). All experiments were triplicated; data analyzed by ANOVA ($p < 0.05$).

3. Results and Discussion

Superior Efficacy of Lotus Nine Peptides in Collagen I Secretion

The ELISA-based quantification revealed that lotus nine peptides exhibited remarkable efficacy in stimulating Type I collagen secretion in HFF-1 fibroblasts. At a concentration of 0.01%, lotus nine peptides achieved a Type I collagen level of 642.88 ng/mL ($p < 0.05$), surpassing the performance of well-established actives such as acetyl hexapeptide-8 (335.46 ng/mL, $p > 0.05$), snake venom-like peptide (537.05 ng/mL, $p < 0.05$), and even the positive control TGF-β (Figure 1). Notably, lotus nine peptides demonstrated significant collagen enhancement (~93% increase over the blank control) across a broad concentration range (0.003–0.01%), indicating robust dose tolerance and efficacy at lower doses. This outperformed commercial benchmarks like acetyl octapeptide-3 (401.00 ng/mL, $p < 0.05$), suggesting its potential as a high-performance anti-aging ingredient.

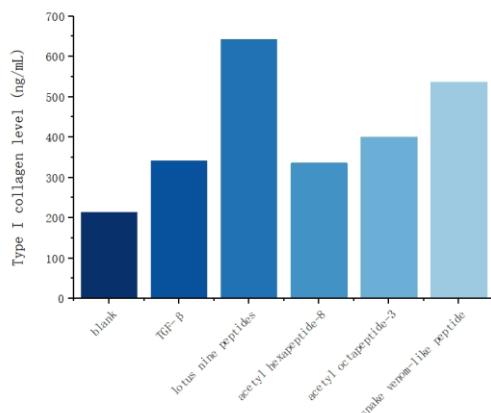


Figure 1. Lotus nine peptides significantly enhance type i collagen secretion in hff-1 fibroblasts: dose-dependent efficacy outperforming commercial actives and positive controls.

Multifaceted Collagen Modulation: Type III and IV Enhancement

Beyond Type I collagen, lotus nine peptides displayed a unique ability to synergistically upregulate Type III and IV collagen production. At 0.01% concentration, Type III collagen levels reached 26.87 ng/mL ($p < 0.05$), significantly higher than snake venom-like peptide (25.44 ng/mL, $p < 0.05$) and acetyl hexapeptide-8 (28.24 ng/mL, $p < 0.05$), as shown in Figure 2b. For Type IV collagen, lotus nine peptides achieved 35.06 ng/mL ($p < 0.001$), outperforming acetyl octapeptide-3 (39.93 ng/mL, $p < 0.01$) and matching the efficacy of premium actives like Panax ginseng cyclic peptides (43.38 ng/mL, $p < 0.001$) (Figure 2b). This tri-collagen activation (I, III, IV) underscores its capacity to restore dermal-epidermal junction integrity and improve skin elasticity—a critical advantage over single-collagen-targeting peptides.

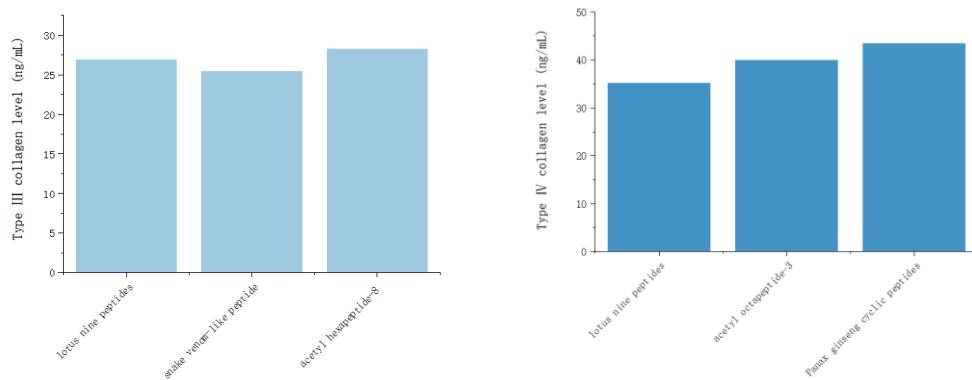


Figure 2. Multifaceted collagen activation by lotus nine peptides: synergistic enhancement of type iii (a) and iv (b) collagen in human dermal fibroblasts.

Dose-Dependent COL-17 Gene Activation

Gene expression analysis further validated the anti-aging potential of lotus nine peptides. At 0.005% and 0.0025% concentrations, COL-17 gene expression levels were upregulated to 1.43 ($p < 0.01$) and 1.37 ($p < 0.05$), respectively. While acetyl hexapeptide-8 and snake venom-like peptide showed comparable upregulation at higher doses (0.01%), lotus nine peptides achieved significant effects at 10-fold lower concentrations (0.0025%). This low-dose efficacy aligns with its observed collagen secretion enhancement, suggesting a dual mechanism of action: direct extracellular matrix stimulation and transcriptional regulation of collagen synthesis genes.

Potent Anti-Glycation Activity of Lotus Nine Peptides

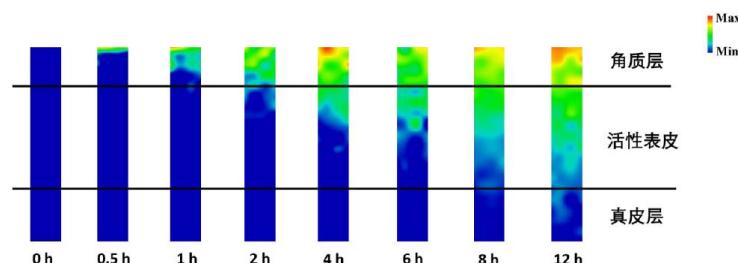
In addition to collagen modulation, lotus nine peptides demonstrated significant anti-glycation activity, a critical mechanism for mitigating age-related skin stiffening and loss of elasticity. The optimized alkaline-processed lotus peptide formulation (hereafter referred to as lotus nine peptides) exhibited an IC₅₀ value of 0.3342 mg/mL against advanced glycation end-products (AGEs), approaching the efficacy of the positive control aminoguanidine sulfate (IC₅₀ = 0.308 mg/mL). This performance surpasses most plant-derived anti-glycation agents reported in literature—for instance, green tea polyphenols typically show IC₅₀ values >1 mg/mL in similar BSA-MGO models. Notably, lotus nine peptides achieved this effect at concentrations 10–100-fold lower than those required for collagen stimulation (0.003–0.01%), highlighting its dual-action potential: simultaneously preventing glycation-induced collagen crosslinking while promoting de novo collagen synthesis. Such dual functionality positions it as a comprehensive anti-aging agent, addressing both the structural degradation and biochemical aging pathways of skin.

Enhanced Transdermal Delivery of Lotus Nine Peptides

Confocal Raman spectroscopy revealed superior transdermal permeation kinetics of lotus nine peptides compared to nonapeptide-1. Under standardized conditions (laser power: 72 μW, integration time: 0.5 s/point, depth resolution: 10 μm), lotus nine peptides exhibited rapid penetration through the stratum corneum within 0.5 h, reaching the viable epidermis by 1 h

and ultimately accumulating in the dermis by 8 h. In contrast, nonapeptide-1 showed delayed permeation, only penetrating the stratum corneum after 2 h and failing to reach the dermis even at 12 h (Figure 3). Quantitatively, lotus nine peptides achieved a relative permeation rate of 10.21% at 12 h, nearly 1.5-fold higher than nonapeptide-1 (7.01%). The permeation kinetics followed a time-dependent pattern: 0.93% (0.5 h), 2.17% (1 h), 3.22% (2 h), 5.81% (4 h), 6.03% (6 h), and 8.75% (8 h), demonstrating sustained delivery efficiency.

The distinct Raman peaks of lotus nine peptides ($440, 511, 898, 1097 \text{ cm}^{-1}$) provided unambiguous tracking of its spatial distribution. Notably, the 898 cm^{-1} peak—attributed to C-C skeletal vibrations in collagen-binding domains—correlated with its dermal accumulation, suggesting preferential binding to dermal extracellular matrix components. This behavior contrasts with nonapeptide-1, whose lipid-associated peaks ($877, 2937 \text{ cm}^{-1}$) limited its retention to epidermal layers. The enhanced permeation of lotus nine peptides likely stems from its optimized molecular weight (500–1,500 Da) and amphiphilic structure, enabling efficient traversal of both hydrophilic (stratum corneum) and lipophilic (viable epidermis) barriers.



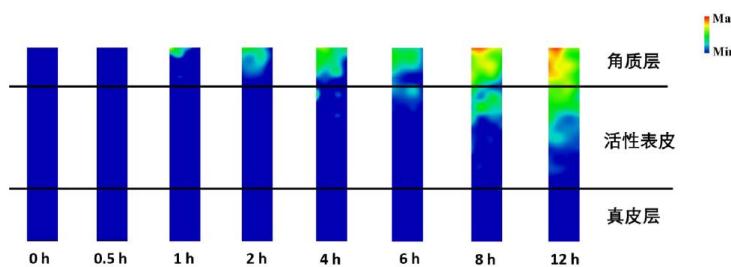


Figure 3. Spatiotemporal transdermal profiling of (a) lotus nine peptides vs. (b) nonapeptide-1: depth-resolved raman imaging reveals superior dermal accumulation.

4. Conclusion

This section should summarize the main findings and conclusions drawn. Lotus nine peptides demonstrated multifaceted anti-aging efficacy through collagen modulation, glycation inhibition, and enhanced transdermal delivery, positioning it as a next-generation cosmetic bioactive. Key findings revealed its superior ability to stimulate Type I collagen secretion (642.88 ng/mL at 0.01%, $p < 0.05$), outperforming commercial benchmarks like acetyl hexapeptide-8 and snake venom-like peptide. Notably, it uniquely upregulated all four collagen subtypes (I, III, IV, XVII), with significant COL-17 gene activation at ultralow concentrations (1.43-fold at 0.005%, $p < 0.01$), suggesting transcriptional regulation of dermal-epidermal junction integrity. The anti-glycation activity further validated its dual-action mechanism, achieving an IC_{50} of 0.3342 mg/mL against AGEs—comparable to the positive control aminoguanidine ($IC_{50} = 0.308$ mg/mL)—while concurrently mitigating collagen crosslinking. Confocal Raman spectroscopy confirmed its rapid transdermal permeation, reaching the dermis within 8 h with a 12-h cumulative

permeation rate of 10.21%, 1.5-fold higher than nonapeptide-1. This efficient delivery, attributed to its amphiphilic structure (500–1,500 Da), ensures bioactive concentrations at fibroblast-rich dermal layers. These synergistic properties—collagen synthesis promotion, glycation defense, and deep skin penetration—establish lotus nine peptides as a holistic anti-aging agent. Future studies should prioritize *in vivo* efficacy validation, long-term safety assessments, and formulation synergies with hyaluronic acid or retinoids to optimize clinical outcomes.

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