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"A brand-new natural plant oils complex with the "Golden-Triangle Concept" of anti-aging effects in multiple dimensions"

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1. Introduction

Plant oils, as natural multi-functional ingredients, have been widely utilized in food, cosmetics, and pharmaceutical products around the world[1]. Whether in nutritional supplementation, wellness preservation, or skincare, these oils are highly valued for their widespread availability, cost-effectiveness, abundance of natural bioactive compounds, and safety[2, 3]. Their role in maintaining skin homeostasis is now increasingly recognized. With advancing age, the skin undergoes progressive degenerative changes, including thinning, roughness, wrinkle formation, dryness, and impaired barrier function[4, 5]. Natural plant oils can exert synergistic anti-aging effects by strengthening the skin barrier, mitigating inflammatory responses, and reducing transepidermal water loss (TEWL), thereby effectively delaying skin aging[6].

Natural plant oils from diverse botanical sources contain distinct bioactive compositions that confer multifaceted anti-aging benefits through specific mechanistic pathways. For instance, perilla oil—enriched with α-linolenic acid, oleic acid, and linoleic acid—demonstrates notable efficacy in mitigating photoaging by suppressing wrinkles, reducing TEWL, and lowering skin erythema index (EI)[7]. However, single plant oils often exhibit limited capacity to simultaneously address multidimensional hallmarks of skin aging. Strategic formulation of the complex of natural plant oils can overcome this limitation by harnessing synergistic interactions among complementary bioactive compounds[8, 9]. Such optimized complexes meet the growing demand for holistic skincare solutions that align with the integrative physiology of aging skin.

This article innovatively proposes the "Golden-Triangle Concept" oil blending, which means creating a complex of natural plant oils that combines balanced and stable unsaturated fatty acids, natural oil-soluble bioactive substances, and aromatic essential oils with sensory pleasure. Plant oils complex POP is a blend of five oil-soluble active substances: Oryza sativa (rice) bran oil, Perilla ocymoides seed oil, Pinus koraiensis seed oil, Pogostemon cablin oil, and Rosmarinus officinalis (rosemary) leaf extract. The content of unsaturated fatty acids in Plant oils complex POP exceeds 80%, mainly by a specific proportion of oleic acid, linoleic acid, and α-linolenic acid. These fatty acids are necessary for the maintenance of epidermal integrity and the water barrier of the skin since they are metabolic precursors of arachidonic acid and prostaglandins in the epidermis and are essential for the regulation of cell division and epidermis differentiation[10]. Linoleic acid has been extensively studied for its role in strengthening the skin barrier[11], while α-linolenic acid is known for its anti-inflammatory effects[12]. The high content of natural vitamin E and oryzanol in oryza sativa bran oil, the unique pinolenic acid in pinus koraiensis seed oil, the patchoulene in pogostemon cablin oil, and the carnosic acid in rosmarinus officinalis extract are natural lipid-soluble bioactive compounds that exert anti-aging effects through antioxidant, anti-inflammatory, and other mechanisms[13-16]. Furthermore, this system, protected by natural antioxidants, serves as an excellent, stable, and high-content supplement of essential fatty acids for the skin.

This study aims to investigate the anti-aging effects of the natural plant oil complex Plant oils complex POP through multidimensional mechanisms. Key aging-related evaluation parameters include skin barrier function, pro-inflammatory factor secretion, skin EI and redness area, skin roughness, desquamation index, skin elasticity and firmness, and wrinkle area. By comprehensively assessing its synergistic effects on skin barrier strengthening, anti-inflammation, soothing, nourishing, tightening, and wrinkle reduction, we demonstrated the distinctive advantages of this natural skincare ingredient complex in combating skin aging.

2. Materials and Methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) culture medium, 10569010, Gibco; Lipopolysaccharides (LPS), LPS25, Sigma-Aldrich; Human IL-6 ELISA kit, EK106, Liankebio.

2.2. Gas chromatography

The sample was subjected to fat extraction using a hydrolysis-ether solution, followed by saponification and methylation under alkaline conditions to generate fatty acid methyl esters. The fatty acid methyl esters were then analyzed by capillary gas chromatography, with fatty acid percentages quantified via the area normalization method.

2.3. Cell culture

The immortalized human keratinocyte cell line HaCaT was acquired from the ATCC (VA, USA). HaCaT cells were maintained in the DMEM culture medium. Cell cultures were incubated at 37°C in a 5% CO2 humidified atmosphere. The medium was refreshed every 48 hours, and subculturing was performed when cells achieved 70-80% confluence.

2.4. Hematoxylin and eosin (H&E) staining

3D human epidermal models were generated by air-liquid interface culture of human primary keratinocytes. After 8-day culture, models were treated with 2% or 5% Plant oils complex POP (18μL/cm²) for 48 hours. Fixed samples were paraffin-embedded, sectioned at 5μm, and stained with H&E for microscopic evaluation, imaged by Leica DM2500 LED.

2.5. Immunofluorescence (IF)

After methanol fixation, tissue sections (5μm) were incubated with primary antibodies at 4°C overnight and secondary antibodies for 1.5 hours in dark. Slides were mounted and imaged by Leica DM2500 LED.

2.6. Enzyme-linked immunosorbent assay (ELISA)

HaCaT cells were seeded in 24-well plates and cultured for 24 hours. The cells were then treated with 10 μg/mL LPS for 24 hours in the presence or absence of 0.005% or 0.0025% Plant oils complex POP. Following treatment, culture supernatants were collected and interleukin-6 (IL-6) concentrations were quantified using a human IL-6 ELISA kit.

2.7. Subject population

The subjects of this study were 18 Chinese healthy female volunteers aged 18-40 years with sensitive skin (lactic acid stinging test score ≥3), exhibiting crow's feet wrinkles graded ≥2 by professional assessment. Exclusion criteria include hyper-sensitive constitution, history of allergic dermatitis, and other dermatological conditions.

2.8. Human efficacy evaluation methods

A split-face, randomized, controlled study design was implemented. Participants applied 5% Plant oils complex POP twice daily (morning and evening), or applied matrix formula as the control, massaging it onto facial skin until fully absorbed. Standardized instrumental measurements were conducted at baseline, day 3, and day 28 post-treatment. Standardized instrumental includes Mexameter MX 18 probe (Courage & Khazaka), VISIA-7 imaging system (Canfield Scientific), Visioscan VC20 Plus system (Courage & Khazaka), and Cutometer MPA 580 probe (Courage & Khazaka).

2.9. Data analysis

The Change Rate was calculated as: (Day 3/Day 28 value - Baseline value) ÷ Baseline value × 100%. The *p*-value (Within-group) indicates the comparison between Day 3/Day 28 values and baseline values within the same group, while the *p*-value (Between-group) represents the comparison between Plant oils complex POP and matrix formula values at a specific time point. The experimental results are expressed as mean±SD. The Shapiro-Wilk test was employed to assess data normality. Normally distributed data were analyzed using paired t-tests, while non-normally distributed data were evaluated with nonparametric tests. *p<0.05, **p<0.01, ***p<0.001.

3. Results

3.1. High Content of Unsaturated Fatty Acids in Plant oils complex POP

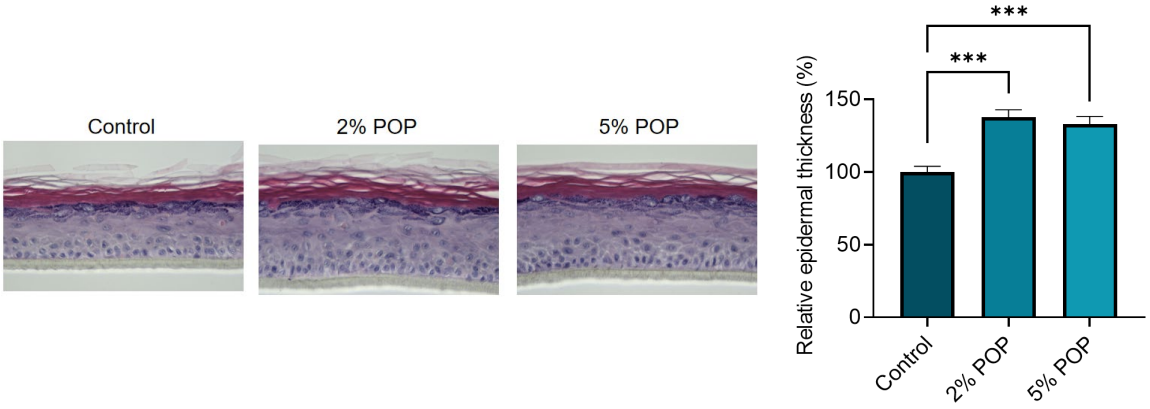
To evaluate the fatty acid composition of Plant oils complex POP, gas chromatography analysis was performed. Results demonstrated that the total unsaturated fatty acid content reached 84.97% (w/w)(Table 1). Specifically, oleic acid, linoleic acid, and α-linolenic acid accounted for 30.63%, 32.86%, and 20.1% respectively (Table 1), with this balanced ratio underpinning its multidimensional anti-aging efficacy.

Table 1. Content of unsaturated fatty acids in Plant oils complex POP.

Composition	Content (*w/w*)
Total unsaturated fatty acids	84.97%
Oleic acid (C18:1)	30.63%
Linoleic acid (C18:2)	32.86%
α-Linolenic acid	20.1%

3.2. Significant Strengthening of Skin Barrier in 3D Reconstructed Human Epidermal Models by Plant oils complex POP

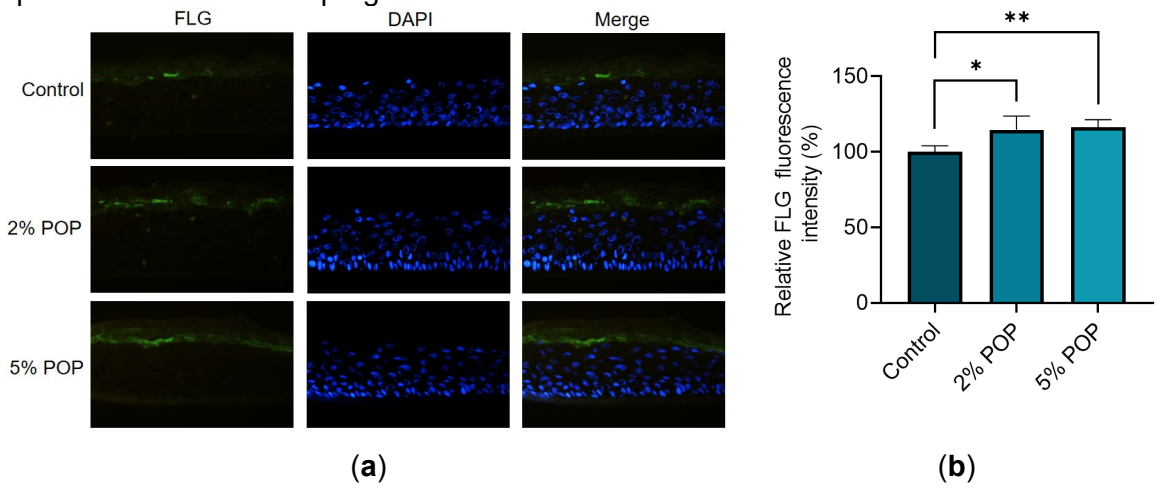
Aged skin typically exhibits epidermal thinning and impaired barrier function[4, 5]. To evaluate the effects of Plant oils complex POP on epidermal thickness and barrier function, 3D reconstructed human epidermal models were employed. Treatment with 2% and 5% Plant oils complex POP significantly increased epidermal thickness compared to untreated controls (Figure 1).



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

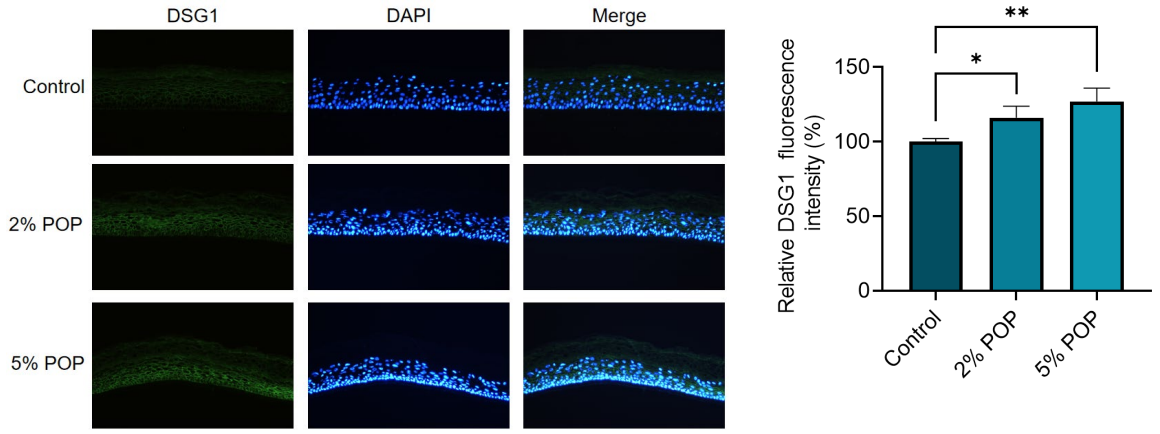
Figure 1. Histological analysis of 3D reconstructed human epidermal models treated with Plant oils complex POP. (a) Representative images of H&E staining showing epidermal morphology (40X); (b) Quantitative analysis of relative epidermal thickness compared with control.

Filaggrin (FLG) mediates epidermal terminal differentiation and facilitates compact stratum corneum formation[17]. Desmosomes constitute critical intercellular junctions in keratinocytes, where desmoglein-1 (DSG1) maintains epidermal integrity through cell-cell adhesion[18]. Immunofluorescence results revealed upregulation of DSG1 and FLG protein levels after 2% and 5% Plant oils complex POP treatment (Figure 2, 3). These findings indicate potent stimulation of epidermal differentiation programs to form the skin barrier.



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

Figure 2. Immunofluorescence analysis of FLG in 3D reconstructed human epidermal models treated with Plant oils complex POP. (a) Representative images of FLG immunofluorescence (40X); (b) Quantitative analysis of relative FLG fluorescence intensity compared with control.



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

Figure 3. Immunofluorescence analysis of DSG1 in 3D reconstructed human epidermal models treated with Plant oils complex POP. (a) Representative images of DSG1 immunofluorescence (40X); (b) Quantitative analysis of relative DSG1 fluorescence intensity compared with control.

Concurrently, ceramides (Cer) constitute 50% of the intercellular lipids in the skin barrier and are an essential component of the skin barrier[11, 19]. Ceramide synthetase 3 (CerS3) is a skin-specific CerS and its content increases with keratinocyte differentiation[20, 21]. CerS3 expression increased in 2% and 5% Plant oils complex POP treated groups (Figure 4), demonstrating enhanced barrier function through promoted ceramide biosynthesis. These findings demonstrate that Plant oils complex POP effectively promotes epidermal integrity, thereby strengthening the skin barrier.

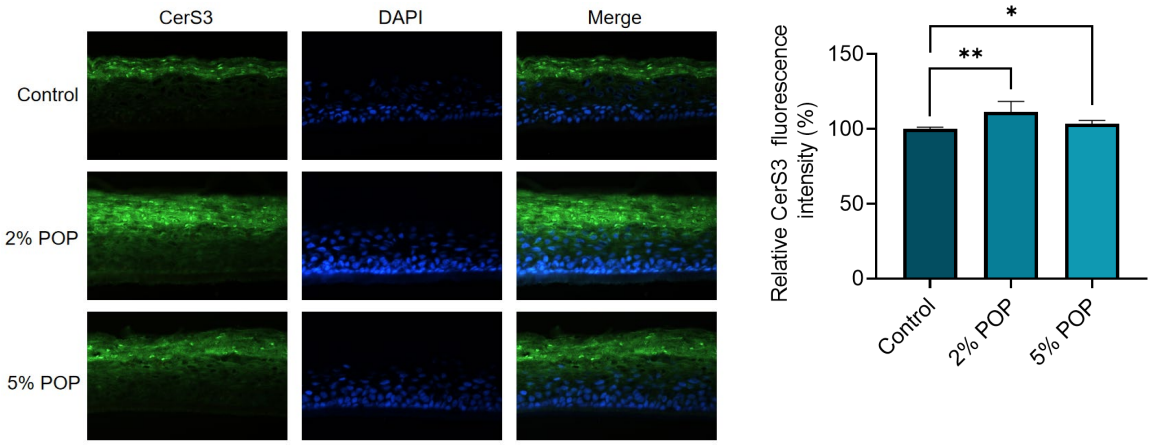


Figure 4. Immunofluorescence analysis of CerS3 in 3D reconstructed human epidermal models treated with Plant oils complex POP. (a) Representative images of CerS3 immunofluorescence (40X); (b) Quantitative analysis of relative CerS3 fluorescence intensity compared with control.

3.3. Reduction of Pro-inflammatory Cytokine Secretion in Keratinocytes by Plant oils complex POP

During skin aging, chronic low-grade inflammation represents a significant pathological characteristic[22]. To systematically evaluate the anti-inflammatory effect of Plant oils complex POP, the study employed an LPS-induced inflammatory keratinocytes model (model control) using HaCaT cells. HaCaT cells were stimulated with 10 μg/mL LPS to investigate the interventional effects of Plant oils complex POP. ELISA results demonstrated that treatment with 0.005% and 0.0025% Plant oils complex POP significantly reduced IL-6 secretion levels in cell supernatants compared to the model group (Figure 5). These findings confirm that Plant oils complex POP effectively inhibits pro-inflammatory cytokine secretion from keratinocytes, thereby alleviating cutaneous skin inflammation.

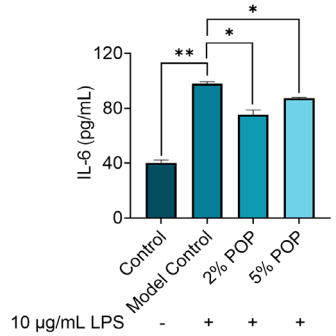


Figure 5. ELISA analysis of IL-6 secreted by HaCaT cells treated with Plant oils complex POP.

3.4. Multidimensional Anti-aging Effects of Plant oils complex POP in Human Efficacy Evaluations

To validate the clinical efficacy of Plant oils complex POP in vivo, this study conducted systematic human efficacy evaluations. Following 3 and 28 days of treatment with 5% Plant oils complex POP, subjects demonstrated significant reductions in skin EI and redness area compared to baseline values (Table 2, 3). More importantly, the improvement in these parameters was significantly greater in the Plant oils complex POP group than in the matrix formula group (Table 2, 3), confirming the exceptional soothing properties of Plant oils complex POP.

Table 2. Effects of Plant oils complex POP versus matrix formula on skin EI (a.u.).

Group	Time Point	Mean	SD	ΔEI	Change Rate	*p*-value (Within-group)	*p*-value (Between-group)
Plant oils complex POP	Baseline	315.85	65.05	-	-		
	Day 3	274.94	56.79	-40.91	-12.95%	＜0.001	0.006
	Day 28	263.26	51.63	-52.59	-16.65%	0.001	0.017
Matrix formula	Baseline	272.46	63.25	-	-	-	-
	Day 3	267.19	59.57	-5.28	-1.94%	0.644	0.006
	Day 28	260.81	63.64	-11.65	-4.28%	0.239	0.017

Table 3. Effects of Plant oils complex POP versus matrix formula on skin redness area (%).

Group	Time Point	Mean	SD	ΔRedness area	Change Rate	*p*-value (Within-group)	*p*-value (Between-group)
Plant oils complex POP	Baseline	0.345	0.030	-	-	-	
	Day 3	0.306	0.036	-0.039	-11.27%	＜0.001	0.003
	Day 28	0.300	0.047	-0.044	-12.86%	＜0.001	0.003
Matrix formula	Baseline	0.314	0.042	-	-	-	-
	Day 3	0.315	0.045	0.001	0.35%	0.963	0.003
	Day 28	0.315	0.039	0.000	0.09%	1.000	0.003

Further analysis revealed that 3 and 28 days of 5% Plant oils complex POP treatment significantly decreased skin roughness and desquamation index compared to baseline (Table 4, 5). The superiority of Plant oils complex POP over the matrix formula in improving skin texture reached statistical significance (Table 4, 5), demonstrating its remarkable nourishing effect.

Table 4. Effects of Plant oils complex POP versus matrix formula on skin roughness (a.u.).

Group	Time Point	Mean	SD	ΔRough-ness	Change Rate	*p*-value (Within-group)	*p*-value (Between-group)
Plant oils complex POP	Baseline	4.471	1.951	-	-	-	
	Day 3	3.169	1.329	-1.30	-29.11%	0.002	0.026
	Day 28	3.019	1.056	-1.45	-32.47%	0.001	0.018
Matrix formula	Baseline	4.333	1.142	-	-	-	-
	Day 3	4.297	1.899	-0.04	-0.83%	0.940	0.026
	Day 28	4.383	1.041	0.05	1.15%	0.895	0.018

Table 5. Effects of Plant oils complex POP versus matrix formula on skin desquamation index (a.u.).

Group	Time Point	Mean	SD	ΔDesquama-tion index	Change Rate	*p*-value (Within-group)	*p*-value (Between-group)
Plant oils complex POP	Baseline	29.849	5.015	-	-	-	
	Day 3	26.394	6.268	-3.45	-11.57%	0.001	0.017
	Day 28	24.576	3.934	-5.27	-17.67%	0.001	0.011
Matrix formula	Baseline	25.119	5.778	-	-	-	-
	Day 3	25.002	4.544	-0.12	-0.46%	0.920	0.017
	Day 28	24.860	4.745	-0.26	-1.03%	0.823	0.011

Regarding skin biomechanical properties, 28-day treatment with 5% Plant oils complex POP significantly increased the skin elasticity parameter R2 while decreasing the skin firmness pa-

rameter F4 (Table 6, 7). These improvements showed statistically significant differences compared to the matrix formula (Table 6, 7), verifying the product's outstanding firming and lifting efficacy.

Table 6. Effects of Plant oils complex POP versus matrix formula on parameter R2 (%).

Group	Time Point	Mean	SD	ΔR2	Change Rate	*p*-value (Within-group)	*p*-value (Between-group)
Plant oils complex POP	Baseline	58.37	8.08	-	-	-	
	Day 28	65.15	7.77	6.78	11.61%	＜0.001	0.014
Matrix formula	Baseline	55.37	8.55	-	-	-	-
	Day 28	56.76	7.16	1.39	2.51%	0.422	0.014

Table7. Effects of Plant oils complex POP versus matrix formula on parameter F4 (mm*s).

Group	Time Point	Mean	SD	ΔF4	Change Rate	*p*-value (Within-group)	*p*-value (Between-group)
Plant oils complex POP	Baseline	5.73	1.03	-	-	-	
	Day 28	4.98	1.17	-0.75	-13.08%	0.004	0.038
Matrix formula	Baseline	6.13	1.14	-	-	-	-
	Day 28	6.19	1.34	0.06	1.01%	0.827	0.038

Most notably, 28-day treatment with 5% Plant oils complex POP resulted in a significant reduction in wrinkle area compared to baseline, with markedly better performance than the matrix formula (Table 8), conclusively demonstrating its superior anti-wrinkle effects.

Table8. Effects of Plant oils complex POP versus matrix formula on skin wrinkle area (%).

Group	Time Point	Mean	SD	ΔWrinkle area	Change Rate	*p*-value (Within-group)	*p*-value (Between-group)
Plant oils complex POP	Baseline	0.087	0.024	-	-	-	
	Day 28	0.071	0.027	-0.017	-19.13%	0.001	0.004
Matrix formula	Baseline	0.078	0.026	-	-	-	-
	Day 28	0.074	0.023	-0.004	-5.20%	0.341	0.004

In conclusion, through its unique "Golden Triangle" formulation system, Plant oils complex POP exhibits significantly better anti-aging efficacy than the matrix formula across multiple skin parameters, including soothing, nourishing, firming, and wrinkle-reducing effects, providing robust scientific evidence for the clinical application of natural plant-derived anti-aging compounds.

4. Discussion

This study systematically validates the multi-dimensional anti-aging effects of the natural plant oil complex Plant oils complex POP through comprehensive in vitro experiments and efficacy evaluation in humans. In vitro, this research employed a 3D reconstructed human epidermal model and keratinocyte assays to demonstrate its remarkable skin barrier-strengthening and reducing pro-inflammatory cytokine secretion capabilities. Human efficacy evaluations confirmed its holistic anti-aging benefits, including decreasing skin EI and redness area, reducing skin roughness, relieving skin desquamation, increasing skin elasticity and firmness, and reducing wrinkle area. With an unsaturated fatty acid content exceeding 80%, including 32% oleic acid, 32% linoleic acid, and 20% α-linolenic acid, this precisely balanced formulation constitutes the core mechanism underlying its multidimensional anti-aging efficacy. Scientific

studies have demonstrated that these unsaturated fatty acids combat skin aging through multiple molecular mechanisms: enhancing ceramide synthesis to strengthen the skin barrier function[23, 24], reducing inflammatory responses to maintain cutaneous homeostasis[12, 25], and improving moisture retention to decrease TEWL[1]. The synergistic interaction of these unsaturated fatty acids enables specific intervention across multiple pathways of cutaneous aging, establishing Plant oils complex POP as a scientifically validated and functionally optimized skincare innovation.

Emerging studies have substantiated that distinct plant oils exhibit characteristic variations in their oleic acid, linoleic acid, and α-linolenic acid ratios, which critically determine their anti-aging efficacy—particularly in skin barrier repair and hydration[26, 27]. For instance, high linoleic acid/oleic acid ratio plant oils demonstrate superior skin barrier repair capabilities, while oleic acid-dominant plant oils exhibit enhanced cutaneous penetration capacity[1, 26]. However, the precise influence of these unsaturated fatty acid ratios on both therapeutic potency and cutaneous tolerability remains contentious, with no established consensus in the field.

To maximize their efficacy in repairing the aging skin barrier, optimizing their formulations has emerged as a critical research focus. Future studies should prioritize investigating how different unsaturated fatty acid ratios influence skin permeability, reparative capacity, and anti-inflammatory effects, while further evaluating their long-term safety profiles and clinical outcomes. This research direction will not only enable more personalized and precise skincare solutions for consumers but also establish a stronger scientific foundation for the application of natural plant oils in dermatological care.

5. Conclusion

Plant oils complex POP contains a high level of nearly stable essential fatty acid, which delivers a comprehensive solution to skin aging through its remarkable skin barrier-strengthening, reducing inflammatory responses, soothing, nourishing, tightening, and wrinkle-reducing properties. These findings conclusively establish the scientific validity and forward-thinking nature of the "Golden-Triangle Concept" oil blending. This multi-dimensional formulation strategy not only inherits the traditional wisdom of natural plant oil applications but also incorporates modern dermatological innovations, establishing a quantifiable and replicable standard for natural plant oil compounding.

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