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*IFSCC 2025 full paper (IFSCC 2025-879)*

## ***“Tailored cosmetic products containing TCM ingredients (Panax ginseng Root Extract) for menopausal women in China”***

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### **1. Introduction**

Menopause is a natural transition stage in a woman's life, marking the shift from the reproductive period to the non - reproductive period. The physiological definition [1] of menopause has amenorrhea as its core, but it also includes some transitional stages before and after, including the menopausal transition period, menopause, and postmenopause. The menopausal transition period refers to the time from the beginning of the decline of ovarian function to amenorrhea. It usually lasts for several years and may start around the age of 40. [2-3] Regarding the age of natural menopause, different scholars' investigations in different countries and ethnic groups have shown certain differences, with a range from 45 to 51 years old. The impact of menopause on women can last for several years or even more than a decade.

Changes in the skin observed during the menopause are similar in fact to those observed during intrinsic and photo-induced ageing. [4] Oestrogen and androgen receptors have been identified in dermal fibroblasts and epidermal keratinocytes. The decrease in oestrogens leads to reduction in collagen I and III and to a decrease in their ratio (III:I ratio). Immediately after the menopause, the decrease in the level of collagen in the skin is fairly rapid and then becomes more gradual: we see around 30% collagen loss in the 5 years following the menopause with a 2.1% decrease per year [5] over a 20 year period. The decreasing collagen leads to a decrease in the skin's elasticity and tone. The reduction in GAGs (glycosaminoglycans) leads directly to a decrease in water content which directly influences the skin's turgescence. [5]

The drop in sebum production accelerates and is then interrupted both on the face and in the scalp. This decrease occurs despite the hyperplasia of the sebaceous gland parenchyma. Later on, xerosis appears, along with a decrease in skin thickness and atrophy. There is less effective irrigation of the skin leading to a decrease in its oxygenation and nutrition. There is also an increase in transepidermal moisture loss values. [6-8]

The impact of menopause on women's skin is extensive but often overlooked. We used a proportional quota sampling method to recruit 2090 postmenopausal women aged 45 - 65 from the French adult population [9]. The research found that menopausal women often face a wide range of skin problems, and the most serious ones are that more than 60% of women are concerned about skin dryness, and more than 45% are worried about deepening wrinkles. These problems are widespread but often not given enough attention. Nearly half of women are unaware that menopause can affect their skin. [9-11] At the same time, there is also a lack of awareness among health professionals, who often underestimate or ignore the impact of menopause on the skin. The presented data are based on French women. There is even less research data on Chinese people, which once again calls for us to educate women and health professionals to increase their awareness of the importance of the impact of menopause on women, so as to improve the quality of life and self - esteem of menopausal women.

In fact, to relieve menopause - related symptoms, the guidelines have proposed some methods to improve and enhance the quality of life. The most common one is a hormone replacement therapy (HRT). Such therapies have many positive effects on the skin of menopausal women. The most common route is oral administration (for 2 - 5 years). Oral hormones are mainly used, after assessment, to relieve some systemic menstrual discomfort, but their positive effects on the skin have also been observed in clinical practice. [12-14] After that, some people specifically studied local skin administration of topical estrogen and found that it can promote collagen production and thicken the epidermis. [15-18] Of course, such hormone replacement therapies are not without corresponding risks and limitations and require professional assessment and guidance. We found that whether it is oral or topical hormone replacement, it may increase the risk of certain diseases. At the same time, its impact on some facial wrinkles or aging indicators is limited. In addition, most Chinese women may not use a large amount of hormonal drugs due to some skin discomfort.

Therefore, today we hope to discuss a solution for improvement in the field of skin care. The skin of menopausal women requires special care and corresponding product design, which is determined by the characteristics of menopausal women's skin. Due to the decrease in hormone levels, there is a reduction in collagen, a decrease in skin plumpness and elasticity, a decrease in facial sebum secretion, and an increase in transepidermal water loss, which often brings symptoms of dryness and discomfort. And because the skin's absorption effect is weakened, its oxygenation and nutrition functions are reduced, which further affects the effectiveness of skin care products. Therefore, a targeted solution is urgently needed.

## 2. Materials and Methods

1. In vitro - Effects on human normal fibroblasts metabolism were assayed by measuring glucose consumption:

Biological models used:

- Normal human dermal fibroblasts (NHDF) BIOalternatives reference PF2

- Normal human keratinocytes NHEK, BIOalternatives reference: K341

Reference molecular: Fetal calf serum (FCS), Supplier code: Gibco, Ref. 10270-098, Tested concentration: 10%

2. In vitro - Fibroblast mitochondrial membrane potential:

Biological models used: Normal human dermal fibroblasts (NHDF) Fb19052002,

Main Reagents: DMEM culture medium (Gibco), PBS (Solarbio), MTT (Sigma), DMSO (Sigma), TGF- $\beta$ 1 (Peprotech), JC-1 mitochondrial membrane potential detection kit (Beyotime).

### 3. In vitro - Procollagen I synthesis:

Reference molecular: Vitamin C, Supplier code: Sigma Ref A4544, Tested concentration: 20 $\mu$ g/mL.

Biological models used: Normal human dermal fibroblasts (NHDF) BIOalternatives reference PF2, used at the 8th passage

### 4. In vivo Evaluation of a Cream formula Containing Ginseng Extract

Population: Gender:Female, N=44,

Main Criteria: Chinese women aged from 45-65 years old, Normal to dry skin, including normal, combine, dry skin, with about 50% sensitive skin (self-declared). Regular users of facial cream with antiaging need. Presenting with lack of sleep (self declared).

Clinical grading by dermatologist on face following:

Forehead wrinkle (Atlas, 2 $\leq$ grade $\leq$ 5), Glabellar wrinkle (Atlas, 2 $\leq$ grade $\leq$ 4), Cheek folds (Atlas, grade $\geq$ 2), Nasolabial fold (Atlas, 2 $\leq$ grade $\leq$ 5), Upper lip wrinkles (Atlas, grade $\geq$ 1), Wrinkle around the corner of the lip (Atlas, grade $\geq$ 2), Ptosis of the lower part of the face (Atlas, 2 $\leq$ grade $\leq$ 4), Horizontal neck folds (Atlas, grade $\geq$ 2), Neck sagging (atlas, 2 $\leq$ grade $\leq$ 5), Whole face plumpness (0-9 scale, 4 $\leq$ grade $\leq$ 6), Skin radiance (0-9 scale, grade $\geq$ 3), Skin smoothness (0-9 scale, grade $\geq$ 3), Presenting with lack of firmness, elasticity, transparency on the face and apple cheek plumpness, evaluated by dermatologist. The global appearance of fine lines, glabellar wrinkles, along with six other types of wrinkles, ptosis of the lower part of the face, and neck sagging were evaluated by means of the L'Oreal Aging Atlas. The severity of plumpness across the whole face and on the apple cheeks, as well as elasticity, smoothness, radiance, transparency, and firmness were assessed using the Griffiths scale.

## Design

Mono - centric, home use, full - face and neck area application, 2 - week washout, 8 - week treatment

- Clinical Assessment by dermatologist at T0, TIMM, T4W and T8W

Whole face plumpness (0 - 9) , skin firmness (0 - 9), apple cheek plumpness (0 - 9), skin transparency (0 - 9), skin elasticity (tactile) (0 - 9), global appearance of fine lines (0 - 9),

- Clinical Assessment by dermatologist at T0, TIMM, T2W, T4W and T8W

skin smoothness (visual) (0 - 9), skin radiance (0 - 9), skin smoothness (tactile) (0 - 9),

- Clinical Assessment by dermatologist at T0, T4W and T8W

Forehead wrinkle (0 - 8 Atlas), wrinkles of the corner of the lips (0 - 8 Atlas), glabellar wrinkles (0 - 6 Atlas), ptosis of the lower part of the face (0 - 6 Atlas), cheek folds (0 - 9 Atlas), horizontal neck fold (0 - 7 Atlas), nasolabial folds (0 - 7 Atlas), neck sagging (0 - 5 Atlas), upper lip wrinkles (0 - 6 Atlas), skin pore (0 - 7 Dermascore Atlas)

- Instrumental measurement T0, TIMM, T4W and T8W

VISIA 7 for standard photos, VC20 for Ser (Roughness)

## 3. Results

In vitro test results of Ginseng root extract

### 1. Glucose consumption and metabolic activity

Treatment		Initial quantity (T0) (mg/ml)	72h						Viability (MTT)
Test compound	Concentration		Glucose (measured quantity) (mg/ml)	Consumed glucose (Initial quantity - Glucose) (mg/ml)	Mean (mg/ml)	sem (mg/ml)	% Control	p(1)	% Control
Control	-	1.102	0.840 0.847 0.809 0.878 0.764 0.839	0.262 0.255 0.293 0.224 0.338 0.263	0.273	0.016	100	-	100
FCS	10%	1.083	0.604 0.587 0.541	0.479 0.496 0.542	0.506	0.019	185	***	155
Ginseng Root extract	0.2 mg/ml	1.145	0.690 0.714 0.718	0.455 0.431 0.427	0.437	0.009	160	***	120

(1): Threshold for statistical significance

\*\*\*: < 0.001, Extremely significant

Under experimental conditions, treatment with FCS on fibroblasts increased significantly intracellular glucose consumption. At a concentration of 0.2mg/mL, Ginseng root extract significantly increased human fibroblasts glucose consumption by 60% after 72H of treatment. This indicates a modulation of metabolic activity in human fibroblasts.

## 2. Fibroblast mitochondrial membrane potential:

Group	JC-1 Aggregate IOD	JC-1 Monomer IOD	Mitochondrial		Mean	SD	P-value	Improvement Rate (vs NC)
			Membrane Potential					
			(JC-1 Aggregate / JC-1 Monomer)					
BC	234021.33	52325.00	4.47		4.87	0.44	/	/
	214862.33	44688.00	4.81					
	262349.00	49088.67	5.34					
NC	95692.00	102154.66	0.94		0.93	0.05	0.000 ##	/
	122443.34	138977.33	0.88					
	127746.00	129757.66	0.98					
PC (TGF-β1)	154617.33	60388.66	2.56		2.75	0.20	0.000 **	195.70%
	176241.33	59393.00	2.97					
	158595.00	57979.67	2.74					
Ginseng Root extract 0.009375%	211730.33	91942.66	2.30		2.21	0.12	0.000 **	137.63%
	197005.67	87820.66	2.24					
	214362.67	103520.66	2.07					

\*\*: P-value < 0.01, Significant

Compared with the BC group, the mitochondrial membrane potential of the NC group decreased significantly, indicating that the stimulation conditions of this experiment were effective. Compared with the NC group, the mitochondrial membrane potential of the PC (TGF-β1) group increased significantly, indicating that the positive control test of this experiment was effective. Compared with the NC group, the mitochondrial membrane

potentials of the samples Ginseng Root Extract -0.009375% increased significantly, with the improvement rates being 137.63%.

### 3. Pro-collagen-1 content

Test compound	Concentration	Procollagen I (ng/ml) (dilution factor adjusted)	Mean (ng/ml)	sem (ng/ml)	% Control (%)	sem	p <sup>(1)</sup>
Control	-	802.5 876.6 873.7	850.9	24.2	100	3	-
Vitamin C	20 µg/ml	7790.4 8043.1 8366.3	8066.6	166.7	948	20	***
Ginseng Root Extract	0.066 mg/ml	948.5 979.9 962.8	963.8	9.1	113	1	*

(<sup>1</sup>): Threshold for statistical significance \*: 0.01 to 0.05, Significant

Under experimental conditions, Vitamin C treatment strongly stimulated Procollagen I synthesis release by cells : those results validate this study. Ginseng extract, increased significantly Procollagen I synthesis at the testing concentration (+ 13% against control).

### Clinical Evaluation

A cosmetic product with Panax ginseng root extracts was tested. After 2 months of using the cream, significant improvements ( $P < 0.05$ ) were seen in skin quality (reduced plumpness and roughness) and aging signs (diminished wrinkles and less lower - face ptosis, Figure. 2) compared to the baseline.

#### Clinical Assessment By Dermatologist

- Compared to baseline, whole face plumpness(visual), apple cheek plumpness(visual), skin elasticity ( skin firmness ( skin transparency (and global appearance of fine lines (are significantly improved at TIMM, T4W, and T8W.

- Skin smoothness and skin radiance are significantly improved at TIMM, T2W, T4W, and T8W.

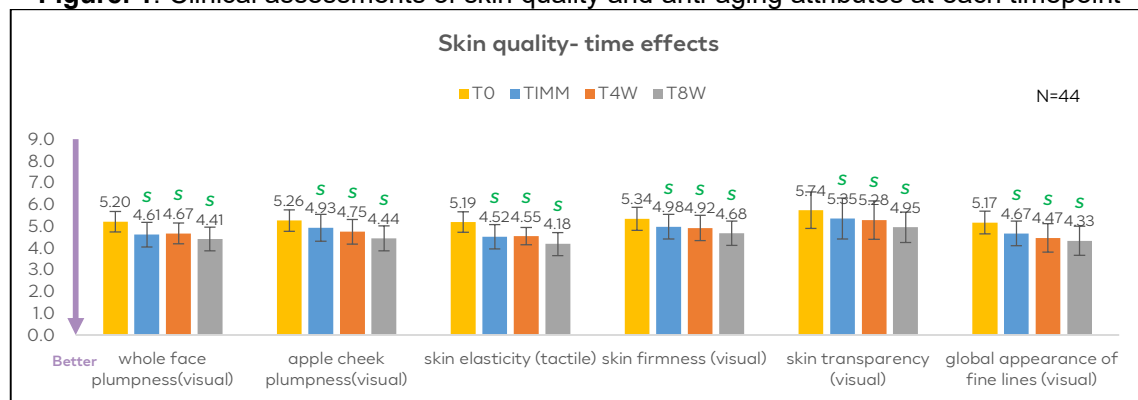
Compared to baseline, forehead wrinkles, glabellar wrinkles, cheek folds, nasolabial folds, upper lip wrinkles, wrinkles of the corner of the lips, ptosis of the lower part of the face, horizontal neck fold, neck sagging, and skin pore by Dermascore are significantly improved at T4W and T8W.

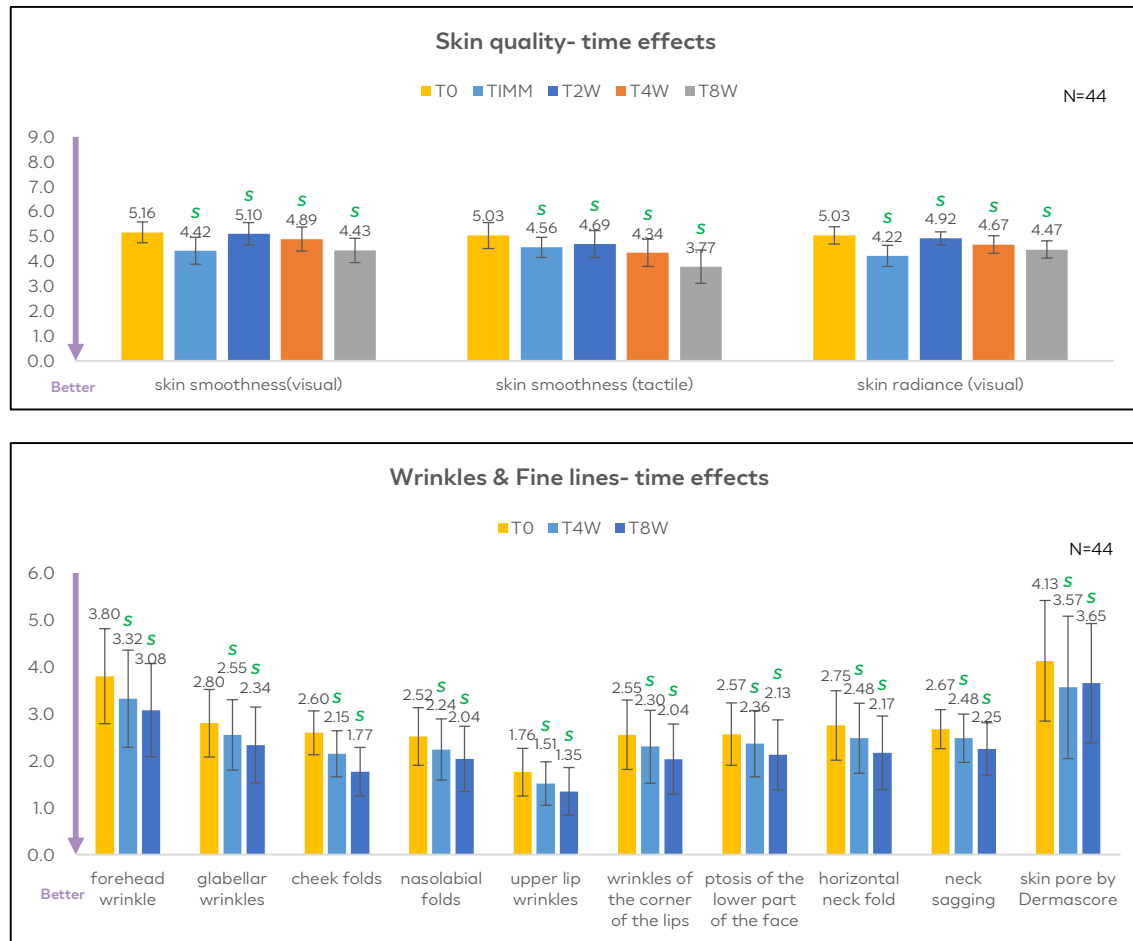
	Attribute	T0 (mean±SD)	T1MM-T0 Delta p <sup>(1)</sup> (Change%)	T2W-T0 Delta p <sup>(1)</sup> (Change%)	T4W-T0 Delta p <sup>(1)</sup> (Change%)	T8W-T0 Delta p <sup>(1)</sup> (Change%)
Skin quality	Whole face plumpness (visual)	5.20±0.47	-0.59*** (-11.35%)	-	-0.53*** (-10.26%)	-0.80*** (-15.28%)
	Apple cheek plumpness (visual)	5.26±0.49	-0.33*** (-6.26%)	-	-0.51*** (-9.72%)	-0.82*** (-15.55%)
	Skin elasticity (tactile)	5.19±0.47	-0.67*** (-12.91%)	-	-0.65*** (-12.47%)	-1.01*** (-19.47%)

	Skin firmness (visual)	5.34±0.54	-0.36*** (-6.81%)	-	-0.42*** (-7.87%)	-0.66*** (-12.34%)
	Skin transparency (visual)	5.74±0.84	-0.39*** (-6.73%)	-	-0.45*** (-7.92%)	-0.78*** (-13.66%)
	Global appearance of fine lines (visual)	5.17±0.53	-0.50*** (-9.67%)	-	-0.70*** (-13.63%)	-0.84*** (-16.26%)
	Skin smoothness(visual)	5.16±0.41	-0.74*** (14.32%)	-0.06* (-1.10%)	-0.27*** (-5.29%)	-0.73*** (-14.10%)
	Skin smoothness (tactile)	5.03±0.52	-0.48*** (-9.48%)	-0.34*** (-6.77%)	-0.69*** (-13.77%)	-1.26*** (-25.06%)
	Skin radiance (visual)	5.03±0.35	-0.82*** (16.25%)	-0.11** (-2.26%)	-0.36*** (-7.22%)	-0.57*** (-11.29%)
Anti-aging	Forehead wrinkle	3.80±1.01	-	-	-0.48*** (-12.56%)	-0.72*** (-19.02%)
	Glabellar wrinkles	2.80±0.72	-	-	-0.25*** (-8.93%)	-0.46*** (-16.56%)
	Cheek folds	2.60±0.47	-	-	-0.45*** (-17.16%)	-0.83*** (-31.87%)
	Nasolabial folds	2.52±0.61	-	-	-0.28*** (-11.01%)	-0.48*** (-18.95%)
	Upper lip wrinkles	1.76±0.51	-	-	-0.25*** (-13.95%)	-0.41*** (-23.51%)
	Wrinkles of the corner of the lips	2.55±0.74	-	-	-0.25*** (-9.96%)	-0.52*** (-20.28%)
	Ptois of the lower part of the face	2.57±0.66	-	-	-0.20*** (-7.96%)	-0.44*** (-17.17%)
	Horizontal neck fold	2.75±0.74	-	-	-0.27*** (-9.90%)	-0.59*** (-21.29%)
	Neck sagging	2.67±0.41	-	-	-0.19*** (-7.14%)	-0.42*** (-15.82%)
	Skin pore by dermascore	4.13±1.28	-	-	-0.56*** (-13.65)	-0.48 (-11.50%)

(\*): Threshold for statistical significance

\*: 0.01 to 0.05, Significant, \*\*: 0.001 to 0.01, Very significant, \*\*\*: &lt; 0.001, Extremely significant

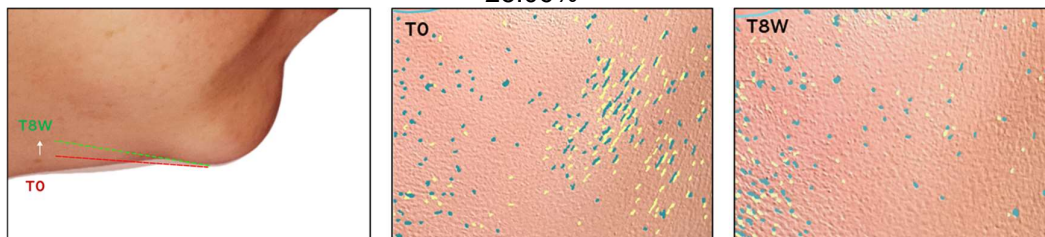
**Figure. 1:** Clinical assessments of skin quality and anti-aging attributes at each timepoint



### Image Capture And Analysis

Compared to baseline, SEr (roughness) by VC20 is significantly improved at T2W, T4W and T8W

**Figure 2 :** Clinical evaluation of a cosmetic product containing Panax ginseng root extracts, after 8-week of use, neck sagging is improved by 15.82%; Roughness is improved by 25.06%



## 4. Discussion

Here we designed a skincare product that combines ginseng root extract with other ingredients to help improve the skin aging phenomenon and relieve dry and uncomfortable skin in menopausal Chinese women. And validated its effect both vitro and vivo.

The first problem to be solved is to deal with the slowdown of energy metabolism in menopausal women. In vitro tests have shown that ginseng root extract can significantly increase the mitochondrial membrane potential of human fibroblasts, improve mitochondrial function, increasing cell energy.

To address the reduction of skin collagen in menopausal women, in vitro tests have proven that ginseng root extract helps to boost procollagen I, helping to reconstruct the dermis structure, and improving skin aging phenomena such as wrinkles, sagging.

Since menopausal women have poor absorption ability and insufficient nutrition, for this ginseng root extract, we have adopted a special phytosome encapsulation technology. The encapsulation with this biomembrane - like microcapsule structure can better assist the absorption and sustained release of the encapsulated substance. Test studies have found that the active substances of ginseng root extract using the encapsulation technology can be continuously released within 8 hours, and the absorption of active ingredients can be up to 7 times [19].

To address the problems of reduced facial sebum secretion and increased transepidermal water loss in menopausal women, we have also combined a series of fatty compounds such as lipid, squalane and buckthorn seed oil, and combined with glycols to achieve multiple moisturizing effects, further promoting the secretion of skin oil and natural moisturizing factors and reducing skin dryness and discomfort.

Through this combination, we tested the clinical effects of the formula containing ginseng root extract on improving skin moisture content and barrier function. Shows that after applying the formula containing ginseng root extract once, it can improve TEWL by more than 40% within one hour and by more than 27% within 8 hours, indication the better the skin barrier function and the less water loss. After applying the formula, the water content of the stratum corneum can be improved by more than 86% within one hour, and the moisturizing effect can last up to 24 hours. An increase in the water content of the skin stratum corneum makes the skin softer and more plump, reducing the appearance of dry lines and further delaying the dryness and discomfort of menopausal women's skin.

To verify the clinical effect of the formula containing ginseng root extract on improving the skin aging signs of menopausal Chinese women, we conducted a single - center research trial on the test formula containing ginseng root extract. In 8 - week experimental period, menopausal Chinese women were recruited and clinically evaluated their facial wrinkles, sagging, and other aging indicators. The test results showed that using the formula rich in ginseng root extract can improve the skin aging phenomenon in multiple dimensions, including various facial wrinkles, sagging, enhance elasticity and firmness of menopausal women.

## 5. Conclusion

Ginseng, a staple in traditional Chinese medicine, is renowned in skincare field for its safety and efficacy. For menopausal women, whose skin absorption declines, the phytosome - encapsulated ginseng formulation is a significant advancement. This encapsulation safeguards ginseng's bioactive elements and improves its transdermal delivery.

In - vitro, ginseng impacts skin cells in multiple ways. It boosts fibroblast mitochondrial function, metabolism, and collagen synthesis, which are key to countering skin aging mechanisms in menopausal women.



The clinical evaluation, conducted on Chinese women aged 46 – 63 y.o, supports these findings. A day cream with ginseng root extracts improved skin quality and reduced aging signs like roughness, wrinkles, and lower - face ptosis in just two months.

Overall, this research offers a new, tailored skincare solution for menopausal women in China. By harnessing ginseng's power, it enhances skin nourishment and effectively reduces visible aging signs, presenting a natural and safe option for maintaining youthful skin.

### Acknowledgments

Herein, acknowledge co-authors Ms. Xiaoyin Zhou, Mr. Yongcai HUANG, Ms. Yang Wang, Ms. Dan Wang, Ms. Kung Chi Hsu for their funding support and strong enthusiasm on completion of this work.

### Conflict of Interest Statement

NONE

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