IFSCC 2025 full paper (IFSCC2025-777)

A Chinese plateau plants-based formulation suitable for Asian skin: gentle, enduring anti-aging effects and mechanism

Keyun Mu[1], Lu Hu[1], Dajuan Miao, Chujie Huang*

SHE LOG (Guangzhou) Biotechnology Co., Ltd, Guangzhou 510080, China

---

1. Introduction

Aging is a natural phenomenon in the process of life development, which involves the degeneration of physiological functions of the body, slowing metabolism, decreasing organ function, changing tissue structure, and changing physical appearance, the most significant manifestation of which is the disappearance of roughness and elasticity of the skin, the appearance of a large number of fine lines, pigmentation, pigmentation leading to skin color change, etc.[1]

Several studies indicated that the barrier strength was weakest in the skin of the East Asian panel compared with the Caucasians and African Americans, and Asian skin maybe more sensitive to exogenous chemicals. The SC, which functions as an important barrier to maintain biological homeostasis[2], is a major factor for controlling penetration, so the observed differences in SC properties may account for the differences in skin sensitivity or skin reactivity that have been reported to be higher in Asian skin[3]. So it is of great concern to find gentle and long-lasting anti-aging solutions to Asian skin. *Tricholoma matsutake* is a wild edible fungus with high nutritional value and special medicinal effects. *T. matsutake* extracts are reported to be rich in polysaccharides, such as β-glucan, which are often used as ingredients in cosmetics.[4] *T. matsutake* also has strong biological activities, including excellent antioxidants, hypocholesterolemic, and anti-aging effects.[5,6]

Bakuchiol is a monoterpene phenolic compound, which has been proven to be a substance with similar biological activity to retinol, with antioxidant, anti-inflammatory, and anti-aging effects.[7-9] Ergothioneine, as a natural anti-aging substance, has been extensively studied.[10,11] Although *T.matsutake*, bakuchiol, and ergothioneine have beneficial effects in anti-aging. Their combined effects on skin aging have not been studied.

Therefore, in this study, *Tricholoma matsutake* extract and its combination with bakuchiol and ergothioneine (hereinafter referred to as TBE) were employed for network pharmacology analysis to explore potential anti-aging mechanisms, 3D skin model and ex vivo skin tissue studies to evaluate structural and molecular changes. Furthermore, TBE was successfully formulated into a face cream, which underwent clinical efficacy testing, consumer perception trials, allergen screening. This integrated approach aimed to elucidate TBE's anti-aging effects and validate its practical application in skincare.

2. Materials and Methods

2.1. Materials

TBE, *tricholoma matsutake* extract combined with bakuchiol and ergothioneine. *Tricholoma Matsutake* Extract, sourced from Yunnan Ingre Biology Technology Co., Ltd., product name: Songling™. Bakuchiol, sourced from Sytheon Corporation, product name: Sytenol® A. Ergothioneine, sourced from Shanghai Ergothioneine Biotechnology Group Co., Ltd., product name: Ergothioneine. The 3D epidermal skin model (EpiKutis®) and human-derived ex vivo skin tissue were sourced from Guangdong Biocell Biotechnology Co., Ltd.

2.2. Network pharmacology

A list of senescence-related targets was retrieved from the GeneCards database (www.genecards.org/) using the keyword“skin aging". The SEA (https://sea.bkslab.org/) and TCMSP (http://tcmspw.com/tcmsp.php) databases were used to identify potential targets for differential metabolites (DMs). Venny (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to intersect the targets of DMs and senescence in order to identify potential targets for DMs against senescence. An analysis of the direct and indirect interactions of these targets was conducted using STRING (www.string-db.org/). The top 20 targets were visualized with PPI network diagrams. Gene Ontology (GO) classification and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were performed using DAVID (https://david.ncifcrf.gov). KEGG pathways and Go terms were visualized using Weishengxin (http://www.bio-informat.ics.com.cn/).

2.3 Treatment of 3D Epidermal Skin Models

The 3D epidermal skin models were transferred to 6-well plates, with 0.9 mL of culture medium added to each well. The models were randomly divided into blank control (BC), negative control (NC), positive control (PC) and TBE group. Each group had 3 replicates and treated according to Table 1.

Table 1. Experimental groups for 3D Epidermal Skin Models testing

Group	Sample	Dosage	Treatment	Model	Indicator	Method
BC	/	/	/	3D Epidermal Skin Models: EpiKutis®	Protein carbonyls	DNPH Fluorescence Staining
NC	/	/	oleic acid: 1 mg/mL UVB: 300mJ/cm^2			
PC	Vitamin C+ Vitamin E	100μg/mL+ 7μg/mL				
Test Group	TBE	Undiluted solution				

2.4 Protein carbonyls evaluation by DNPH Fluorescence Staining

After BSA Blocking, DNPH Stimulation, Primary Antibody Incubation and Secondary Antibody Incubation, wash 3 times with PBS buffer (5 min each). Remove residual PBS with absorbent paper and seal with anti-fade medium. Capture fluorescence images within 24 h using a fluorescence microscope .

2.5 Treatment of Human Ex Vivo Skin Tissue

The ex-vivo human skin was donated by volunteers who have signed an informed

consent form. Biopsy materials were obtained in accordance with Chinese law and in compliance with the requirements of Local Ethics Committees.

Freshly obtained skin tissue was immersed in 75% ethanol for 30 s, followed by 3 washes with sterile PBS buffer. The skin was then cut into 24±2 mm², expands with the epidermal side facing upward and the dermal side facing downward in culture molds. The models were transferred to 6-well plates, with 3.7 mL of culture medium added per well. Cultures were maintained in a 37℃, 5% CO_2 incubator with daily medium replacement.

After 2 days of pre-culture, skin explants were subjected to irradiation and topical treatment according to the experimental groups (Table 2).

Then, tissue samples were used for Tissue Morphology Analysis, Immunohistochemical (IHC) Staining and Immunofluorescence (IF) Assay along with images captured and analyzed.

Table 2. Experimental groups for ex vivo skin tissue testing

Group	Sample	Dosage	Treatment	Model	Indicator	Method
BC	/	/	/	Human Ex Vivo Skin Tissue	Tissue morphology Elastin Collagen Ⅰ Collagen III Collagen IV Collagen XVII	H&E IHC IF
NC	/	/	UVA: 30J/cm²			
PC	Vitamin C+ Vitamin E	100μg/mL+ 7μg/mL	UVB: 50mJ/cm²			
Test Group	TBE	Undiluted solution				

2.6 Allergen Screening

The TBE cream was analyzed for potential allergens through GC-MS Analysis and HPLC-MS/MS Analysis.

2.7 Clinical Study

2.7.1 Ethical Compliance

At the study's outset, written informed consent (approved by the institutional ethics committee) was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki and complied with Chinese regulatory and legal requirements.

2.7.2 Study Design

Participants: 31 East Asian female subjects (aged 35-46 years) with sensitive skin were enrolled.

Intervention: Daily application of TBE cream for 90 consecutive days.

Objective Measurements: Skin hydration (corneometer), TEWL, Elasticity (R2, R7), Firmness (F4), Skin luminance, Sebum production, Skin colorimetry, etc.

Image Analysis: Skin roughness, Skin density, Jawline angle, Wrinkle severity, etc.

Subjective Assessments: Dermatologist evaluations of Safety and efficacy grading, Self-assessment questionnaires on Safety and efficacy.

3. Results

3.1 Network pharmacology analysis

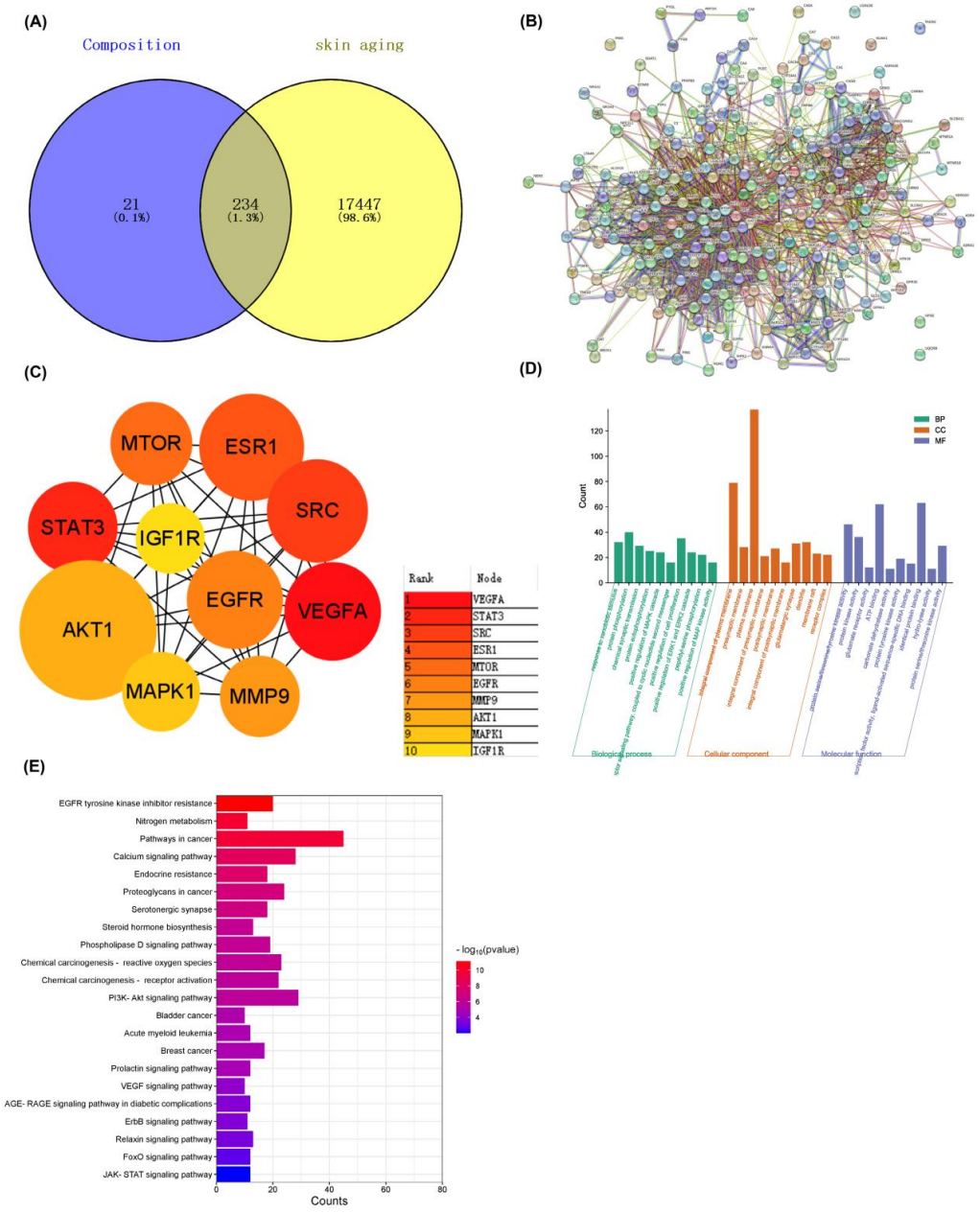


Figure 1. Network pharmacology analysis of "disease-metabolite-target"interaction.(A) Venn chart showed the intersection of targets of neuroinflammation and DMs. (B, C) PPI represented interacion between 234 common targets, of which 10 core targets were shown. (D, E) Ten GO terms in biological processes, cellular components, and molecular function, and the top 30 KEGG pathways of 234 targets were presented.

To investigate the potential mechanisms of TBE against skin aging, a network of interactions based on metabolomics and network pharmacology is developed. 234 common targets are identified by intersecting 17681 skin aging targets with 255 targets associated with *T.matsutake* compositions (Figure 1A). Analyzing potential interactions between targets, PPI analyses are performed on 234 targets (Figure 1B). Ten targets with the highest levels, including VEGFA, STAT3, SRC, ESR1.MTOR, EGFR, MMP9, AKT1, MAPK1, and IGF1R (Figure 1C), are probably the hub targets of TBE against skin aging. For 234 targets, GO annotation and KEGG enrichment analyses are performed using DAVID. Figure 1D displays

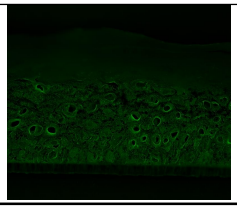
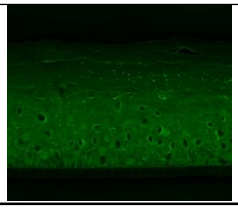
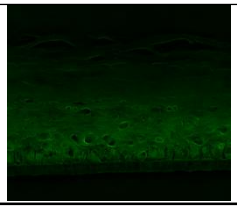
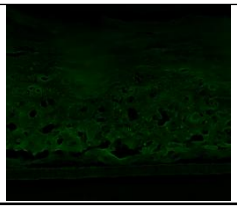
10 key terms for biological processes, cellular components, and molecular function. In most cases, these targets are found in plasma membrane, raft membrane, postsynaptic membrane, and dendritic spine, and involved in xenobiotic resistance, protein phosphorylation and other biological processes. The targets of these studies are enriched in protein kinase activity, ATP binding activity, protein tyrosine kinase binding activity, protein serine/threonine kinase activity, and so on. Based on KEGG, the pathways influenced by *T. matsutake* composition include nitrogen metabolism, calcium signaling, phospholipase D signaling, Pl3K-Akt signaling, prolactin signaling pathways, and so on.

3.2 Protein carbonyls evaluation in 3D Epidermal Skin Models

The 3D epidermal skin model was employed to assess protein carbonyls. DNPH staining was performed to detect protein carbonyls, followed by fluorescence microscopy imaging (Table 3). Integrated Optical Density (IOD) Reflects protein carbonyls content, higher IOD represent elevated protein carbonyls (Figure 2).

$$\text{Inhibition Rate } (\%) = \frac{\text{NC(IOD)} - \text{TBE(IOD)}}{\text{NC(IOD)}} * 100\%$$

Table 3. DNPH Staining Results for Protein Carbonyls

Test	BC	NC	PC	TBE
Protein Carbonyls				

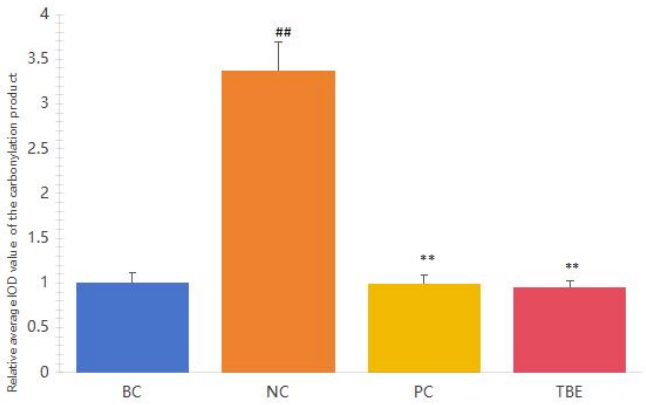


Figure 2. Relative IOD Values for Carbonylated Proteins. The *t-test* was used for analysis. Compared with BC group (blank control), $p < 0.05$ was indicated as $^{\#}$, $p < 0.01$ was indicated as $^{\#\#}$. Compared with NC group (negative control), $p < 0.05$ was indicated as *, $p < 0.01$ was indicated as **.

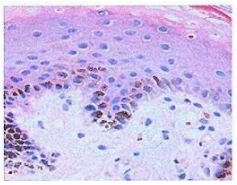
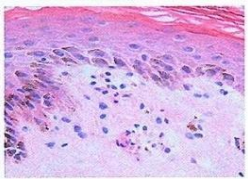
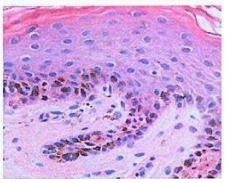
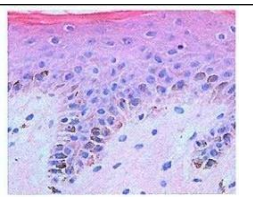
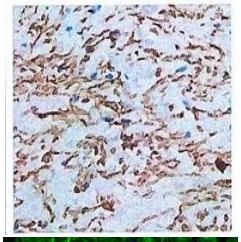
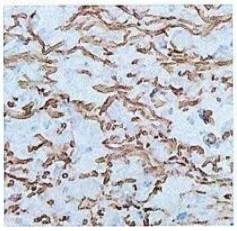
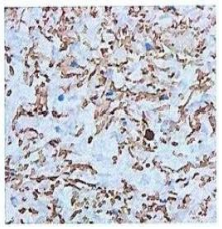
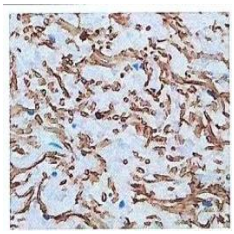
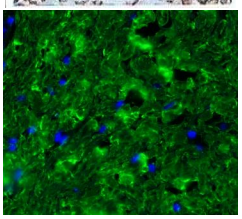
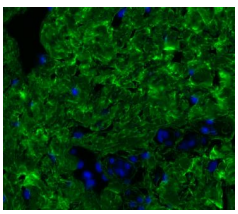
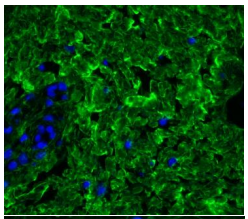
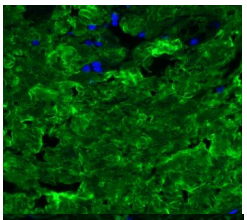
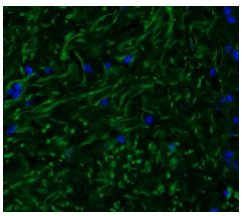
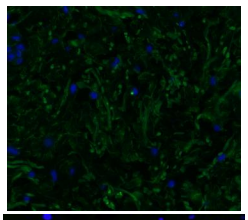
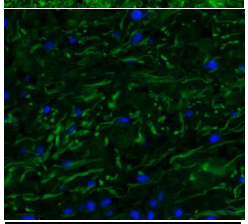
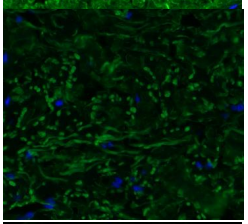
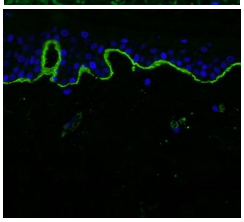
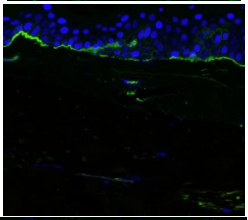
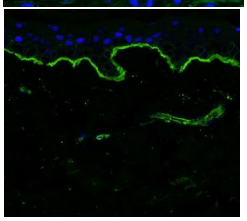
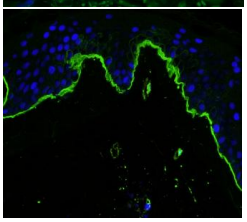
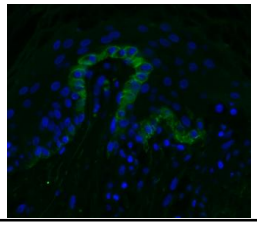
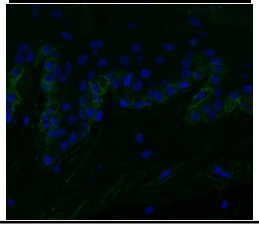
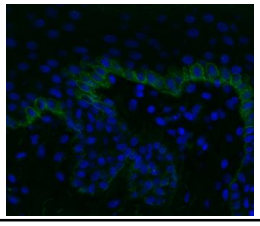
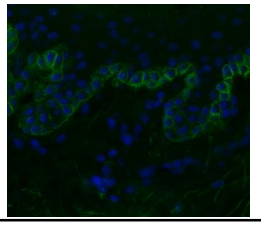
Compared to the BC group, the NC group showed a significant increase in protein carbonyls content, confirming the efficacy of induction model. The PC group exhibited significantly reduced protein carbonyls levels versus NC, validating the reference compound's protective activity. TBE treatment achieved 71.81% inhibition of protein carbonyls relative to NC.

3.3 Anti-aging Efficacy Evaluation in Ex Vivo Skin Tissue

Using human ex vivo skin tissue as the test model to evaluate the anti-aging efficacy of TBE. Evaluation indicators are Epidermal live cell layer thickness, Elastin content and Collagen(I, III, IV, XVII) content.

$$\text{Increase Rate } (\%) = \frac{\text{TBE(IOD)} - \text{NC(IOD)}}{\text{NC(IOD)}} * 100\%$$

Table 4. The images of epidermal live cell layer thickness, elastin and Collagen(I, III, IV, XVII) in Ex Vivo Skin Tissue test

Indicator	BC	NC	PC	TBE
Epidermal live cell layer thickness				
Elastin				
Collagen I				
Collagen III				
Collagen IV				
Collagen XVII				

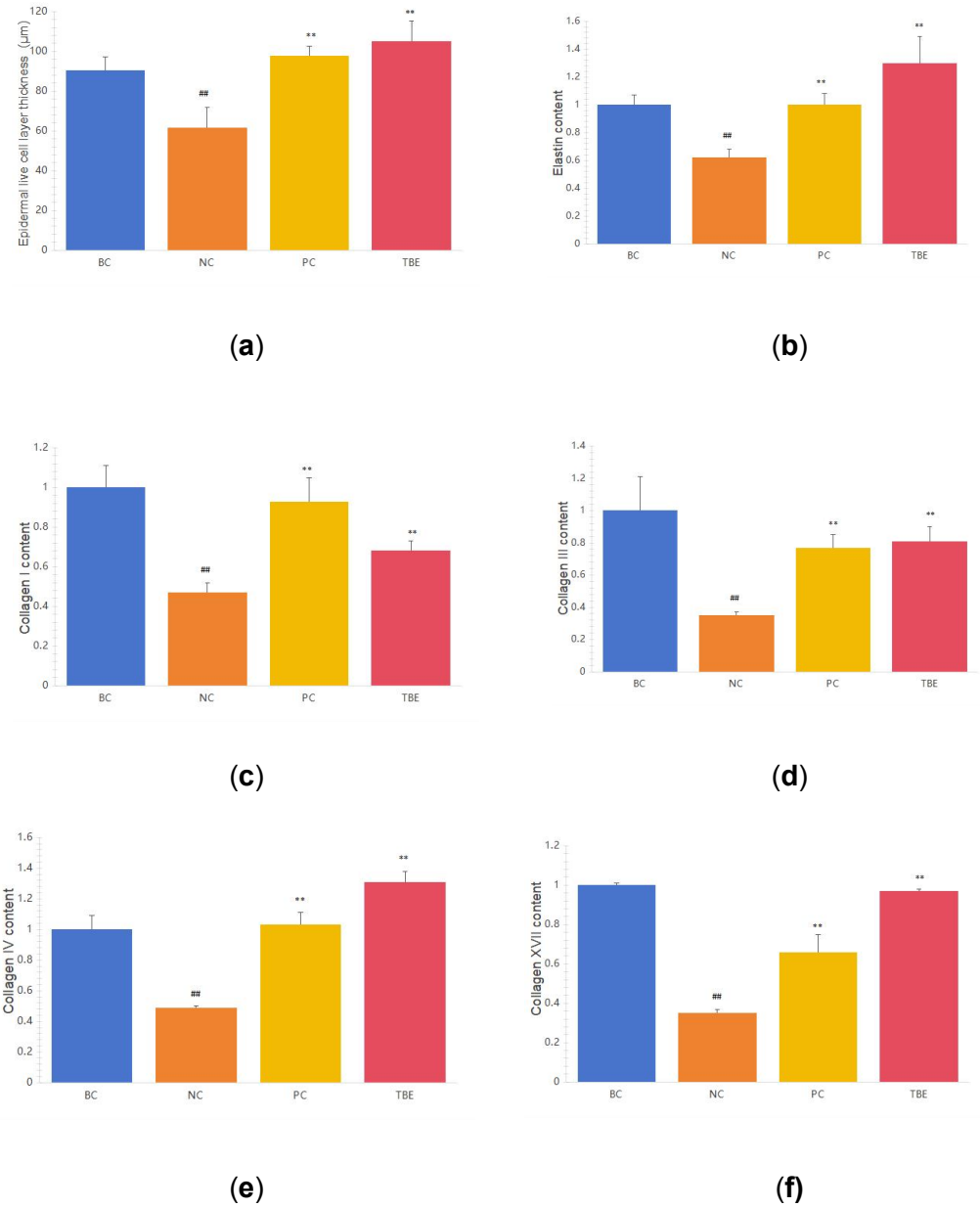


Figure 3. Quantitative analysis of epidermal live cell layer thickness, elastin and Collagen(I, III, IV, XVII) in Ex Vivo Skin Tissue test. a. Epidermal live cell layer thickness; b.Elastin content; c. Collagen I content; d. Collagen III content; e.Collagen IV content; f.Collagen XVII content. The *t-test* was used for statistical analysis. Compared with the BC group (blank control), $p < 0.05$ was indicated as $^{\#}$, $p < 0.01$ was indicated as $^{\#\#}$. Compared with the NC group (negative control), $p < 0.05$ was indicated as *, $p < 0.01$ was indicated as **.

Compared to the BC group, the NC group showed a significant decrease in epidermal live cell layer thickness, elastin content and Collagen (I, III, IV, XVII) content, confirming the efficacy of induction model. The PC group exhibited significantly increased epidermal live cell layer thickness, elastin content and Collagen (I, III, IV, XVII) content versus NC, validating the reference compound's protective activity. Ex Vivo Skin Tissue studies revealed that TBE had a significant increase in epidermal live cell layer thickness, elastin content and Collagen (I, III, IV, XVII) content, with increase rates of 70.64%, 109.68%, 44.68%%, 131.43%, 167.35% and 177.14%.

3.4 Allergen Detection

According to GC-MS and HPLC-MS/MS analysis results, no detectable levels of 26 common fragrance allergens were found in TBE cream, including Limonene, Benzyl Alcohol, Linalool, Methyl 2-Octynoate, Citronellol, ect.

3.5 Human Efficacy Tests of TBE Cream

3.5.1 Equipment Evaluation

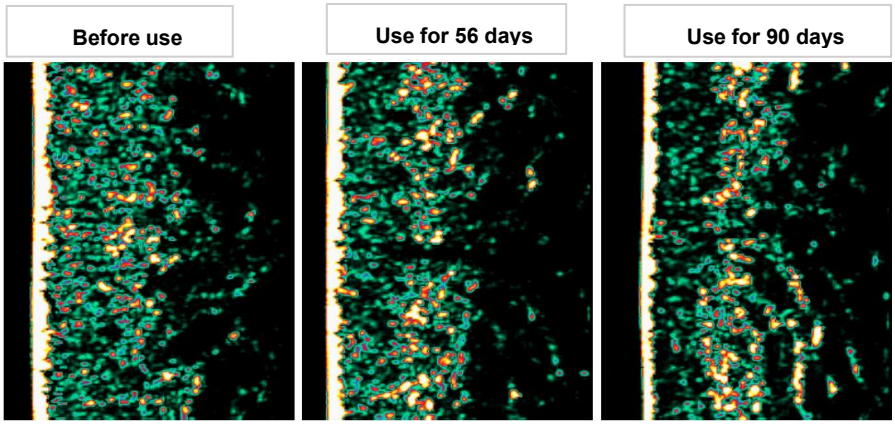


Figure 4. Ultrasound probe of subject No. 11. Areas with white and light yellow colors indicate skin regions with strong echo reflection, such as collagen, elastin, connective tissue, etc. More white and light yellow colors mean bigger skin density value.

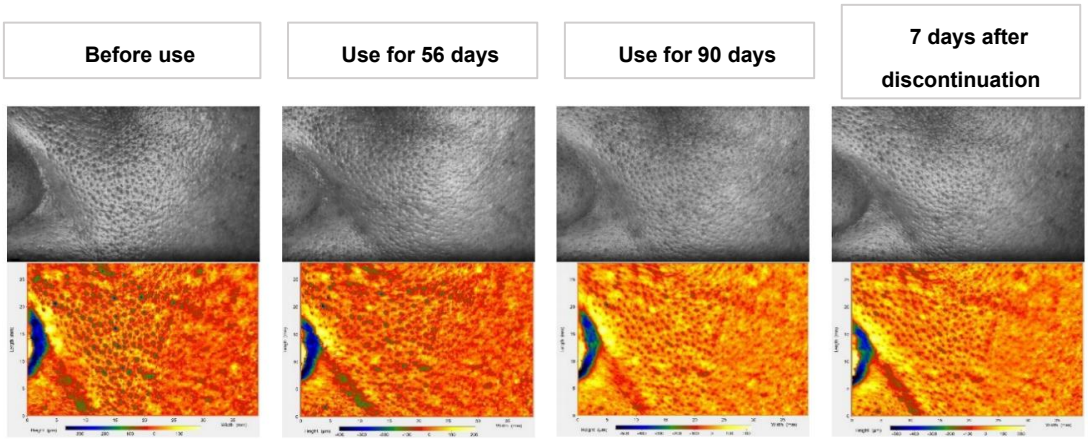


Figure 5. PRIMOS-CR (cheek wrinkles) of subject No. 05. Color change from blue to yellow indicates the wrinkles become shallower.

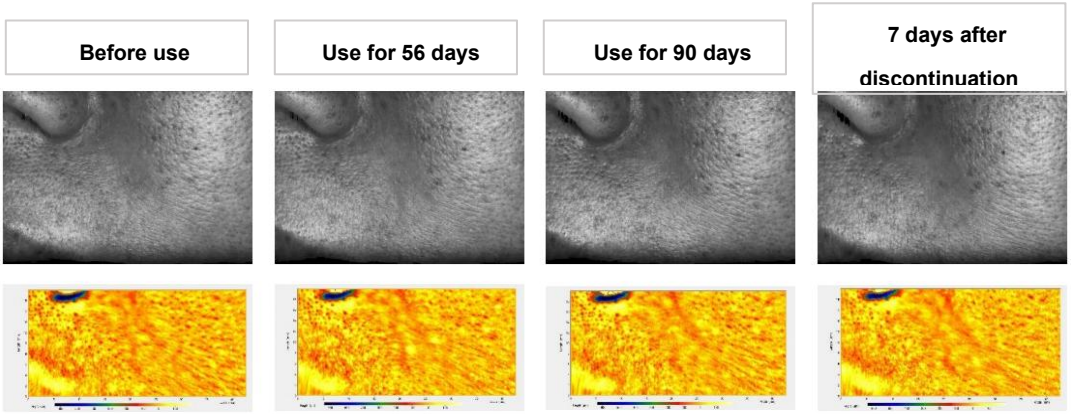


Figure 6. PRIMOS-CR (nasolabial folds) of subject No. 13. Color change from blue to yellow indicates the wrinkles become shallower.

Vivo experiments showed that the formulation had firming and wrinkle-reducing effects, also improve skin glossiness, density and color. After a 7-day discontinuation, there was still an effect on improving wrinkles, indicating its long-lasting performance and do not rebound.

3.5.2 Subjective Evaluation

A total of 31 Asian female subjects (aged 35-46, East Asian skin type, residing in Guangzhou) with sensitive skin were observed. Dermatologist evaluations confirmed the TBE cream's significant efficacy in wrinkle reduction and skin firming(Table 5). No adverse reactions were observed in any of the 31 subjects and none of the 31 subjects reported discomfort during or after product use. 100% of participants agreed that TBE cream was gentle (non-irritating) and suitable for sensitive skin.

Table 5. Dermatologist Efficacy Assessment Results.The *t-test* was used for statistical analysis. Compared with Before Using TBE Cream , $p < 0.05$ was indicated significant difference, $p \geqslant 0.05$ was indicated was indicated no significant difference.

Indicator	Time Point	Before Using TBE Cream (Mean Value)	After Using TBE Cream (Mean Value)	Change Rate	*p*-value
Nasolabial Folds	56 days	4.6	4.0	-13.04%	<0.001**
	90 days	4.6	3.8	-17.39%	<0.001**
	after a 7-day discontinuation	4.6	3.6	-21.74%	<0.001**
Marionette Lines	56 days	3.6	3.2	-11.11%	<0.001**
	90 days	3.6	3.0	-16.67%	<0.001**
	after a 7-day discontinuation	3.6	2.8	-22.22%	<0.001**
Skin Firmness (Full Face)	56 days	4.8	4.4	-8.33%	<0.001**
	90 days	4.8	4.2	-12.50%	<0.001**
	after a 7-day discontinuation	4.8	3.8	-20.83%	<0.001**

4. Discussion

This study explored the potential of TBE combination in anti-aging skincare. Through network pharmacology, we identified potential mechanisms underlying TBE's anti-aging effects. Evaluations using 3D epidermal models and ex vivo skin tissue demonstrated TBE's ability to modulate key aging-related biomarkers. Furthermore, a TBE-enriched cream was developed and clinically tested, showing significant improvements in wrinkle reduction, skin firmness, skin luminosity, barrier function.

Notably, the formulation exhibited excellent safety and tolerability, making it particularly suitable for Asian skin types, especially Chinese consumers with sensitive skin. These findings highlight TBE's promise as a multi-target, holistic anti-aging ingredient.

5. Conclusion

Integrated network pharmacology, 3D skin models, and ex vivo studies revealed that TBE combats skin aging through molecular mechanisms (modulation of external stress

response pathways and regulation of protein phosphorylation cascades) and structural improvements (Epidermal thickening, DEJ reinforcement, Dermal rejuvenation).

The above research indicates that we have successfully constructed a Chinese plateau plants-based formulation, which has good anti-aging effects, no rebound and no adverse reactions. This formulation truly achieves efficiency, long-lasting performance, and gentleness effects, offering a novel and promising anti-aging solution for Asian sensitive skin, especially Chinese consumers with sensitive skin.

6. Reference

[1] Tobin DJ. Introduction to skin aging.J Tissue Viability. 2017;26(1):37-46.

[2] Meguro S, Arai Y, Masukawa Y, Uie K, Tokimitsu I. Relationship between covalently bound ceramides and transepidermal water loss. Arch Dermatol Res 2000;292:463-8.

[3] Kompaore F, Marty JP, Dupont C, In vivo evaluation of the stratum corneum barrier function in blacks, Caucasians and Asians with two noninvasive methods. Skin Pharmacol 1993;6:200-7.

[4] Hoshi H, Yagi Y, Iijima H, Matsunaga K, Ishihara Y, Yasuhara T.Isolation and characterization of a novel immunomodulatory alpha-glucan-protein complex from the mycelium of Tricholoma matsutake in basidio mycetes. J Agric Food Chem. 2005;53(23):8948-8956.

[5] Choi J, Jin M, Lee JK, et al. Control of cytokine gene expression by PG101, a water-soluble extract prepared from Lentinus lepideus. Biochem Biophys Res Commun.2006;339(3):880-887.

[6] Zha Z, Wang S-Y, Chu W, et al. Isolation, purification, structural characterization and immunostimula tory activity of water soluble polysaccharides from Lepidium meyenii. Phytochemistry.2018;147:184-193.

[7] Adhikari S, Joshi R, Patro BS, et al. Antioxidant activity of bakuchiol: experimental evidences and theoretical treatments on the possible involvement of the terpenoid chain. Chem Res Toxicol.2003;16(9):1062-1069.

[8] ThomaA, LightfootAP.NF-kB and inflammatory cytokine Signalling:role in skeletal muscle atrophy. Adv Exp Med Biol. 2018;1088:267-279.

[9] Chaudhuri RK, Bojanowski K. Bakuchiol: a retinol-like functional compound revealed by gene expression profiling and clinically proven to have anti-aging effects. Int J Cosmet Sci. 2014;36(3):221-230.

[10] Paul BD. Ergothioneine: a stress vitamin with anti-aging, vascular,and neuroprotective roles? Antioxid Redox Signal. 2022;36(16-18):1306-1317.

[11] Liu HM, Tang W, Wang XY, Jiang J, Zhang W, Wang W. Safe and effective antioxidant: the biological mechanism and potential path-ways of ergothioneine in the skin. Molecules. 2023;28(4):1648.