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ORIGINAL RESEARCH

A Multidimensional Study Integrating Traditional Chinese Medicine and Network Pharmacology: Exploring the Skin Repair and Anti-Aging Effects of a Specific Combination of Five-Colored Plants

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Background: Skin repair and anti-aging have long been central themes in dermatological and cosmetic science. Rooted in thousands of years of empirical knowledge, Traditional Chinese Medicine (TCM) literature contains systematic records of medicinal plants, many of which have been historically applied to improve skin health.

Objective: Based on the theoretical framework of the Five Elements, this study formulated a specific combination consisting of five chromatically representative medicinal plants—mung bean (green), white truffle (white), black truffle (black), chrysanthemum (yellow), and Lycium barbarum (red)—to investigate their potential synergistic anti-aging and skin regenerative effects.

Methods: The five-colored plants combination was analyzed by network pharmacology, followed by a scratch test of human fibroblasts, β-galactosidase staining, and ex vivo skin model.

Results: The network pharmacological analysis identified 27 core components and 636 targets, including CBS, CDK5, and AKR1B10, which are involved in skin anti-aging and repair. In vitro experiments indicated that at 0.5% concentration, the five-colored plant combination achieved a fibroblast wound healing rate of 59.89% after 24 hours, compared to the negative control group, which was 22.83% higher than that of individual raw materials. In the β-galactosidase staining of human fibroblasts, the blue-stained area in the experimental group was 0.777%, representing a 76.32% reduction compared to the negative control. In the in vitro skin model, treatment with the five-colored plant combination significantly increased the thickness of the epidermal living cell layer, collagen fiber content, and type IV collagen content, with respective increases of 28.16%, 49.12%, and 100.00%. Histological analysis revealed a significant increase in epidermal thickness and a more pronounced the dermal-epidermal junction (DEJ) ridge structure.

Conclusion: This study showed that the five-colored plant combination consisting of mung bean, white truffle, black truffle, chrysanthemum, and Lycium barbarum can repair the skin and delay aging.

Keywords: traditional Chinese medicine, five-colored plants combination, skin repair, anti-aging

Introduction

Traditional Chinese medicine (TCM) has a history of about 5000 years in China, with its theoretical framework having been established for over 2500 years. Many Chinese medicine classics have been published, such as "Huangdi Neijing (Huangdi's Canon of Medicine)" and "Shanghan Zabing Lun (Treatise on Cold Damage and Miscellaneous Diseases)".[1] Among these classics, the "Suwen" and "Lingshu" in Huangdi Neijing reported that all natural phenomena can be divided into "Yin" and "Yang". These two aspects are opposed, complementary, and interdependent. In addition, the Five Elements framework assumes that everything in the universe is composed of five basic elements, namely wood, fire, earth, metal, and water.[2,3] These elements can match the inside and outside, parts and whole of the body, including internal organs of the body (liver, heart, spleen, lung, and kidney), and explain the relationship between internal organs and their physiological and pathological characteristics for effective diagnosis and drug treatment.[4,5] The five elements

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have five matching colors, each of which is associated with specific organs and elemental forces: green is associated with the liver and wood, red with the heart and fire, yellow with the spleen and earth, white with the lungs and metal, and black with the kidneys and water, as displayed in Figure 1. TCM adopts complex treatment methods and herbal formulas to solve various medical problems.[6,7]

In addition to the therapeutic applications of TCM, Chinese herbal medicines have also been used as cosmetics. Sun Simiao, a famous medical scientist in the Tang Dynasty, wrote two books, "Beiji Qianjin Yaofang (Valuable Prescriptions for Emergency)" and "Qianjin Yifang (A Supplement to the Essential Prescriptions Worth a Thousand metal)", which recorded multiple beauty secrets. Li Shizhen's Compendium of Materia Medica in the Ming Dynasty summarized the traditional Chinese medicines used for anti-aging, skin care, and beauty.[8] Many combinations of plants are used in skin care cosmetics based on the beauty formulas in these classic Chinese medicines. For example, seven components in Chi-Bai-San inhibit tyrosinase activity through synergistic action, weaken melanin synthesis in vitro and in vivo, and promote skin whitening.[9] Sijunzi decoction (Ginseng Radix et Rhizoma, Atractylodis macrocephalae Rhizoma, Poria, and Glycyrrhizae Radix et Rhizoma) has been found to mitigate skin aging by acting on multiple targets.[10] In Shennong's Classic of Materia Medica, Qi-Bai-San was reported to whiten the skin, while Angelica sinensis can promote skin growth and exerts a moisturizing effect.[11] Furthermore, many plants or combinations of plants are used in skin beauty care.[12–15] Different plants contain various active ingredients and have many skin care effects. For example, tea tree oil can resist aging and has a certain effect on acne. Glycyrrhiza uralensis Fisch has anti-inflammatory properties and can relieve the skin.[14] A variety of plant combinations can be applied in skin care, exhibiting whitening, moisturizing, anti-wrinkle, and anti-inflammatory properties.

The factors leading to skin aging can be divided into internal and external factors. Under the influence of these factors, the skin, our largest immune organ, gradually loses its physiological function and structural integrity.[16] Skin aging is defined as exogenous aging, which is mainly characterized by reduced skin elasticity, skin roughness, and thickening due to poor lifestyle habits, such as the frequent use of cosmetics or smoking, as well as environmental influences such as ultraviolet rays and air pollution.[17] As age increases, immune function declines and leads to various changes in microenvironments, including oxidative stress, inflammation, and cell aging. These changes result in fine lines, loss of elasticity, and thinning of the epidermis, which are the manifestations of endogenous aging.[18,19] Many methods have been developed to delay skin aging by addressing these factors. This study primarily discusses endogenous aging. Colored plants are often used individually in skin care cosmetics to delay skin aging,[15] but few studies have explored the efficacy of plant combinations fulfilling the five colors and five elements. Therefore, a selection of botanicals was meticulously curated from a vast array, prioritizing their efficacy in retarding skin senescence and

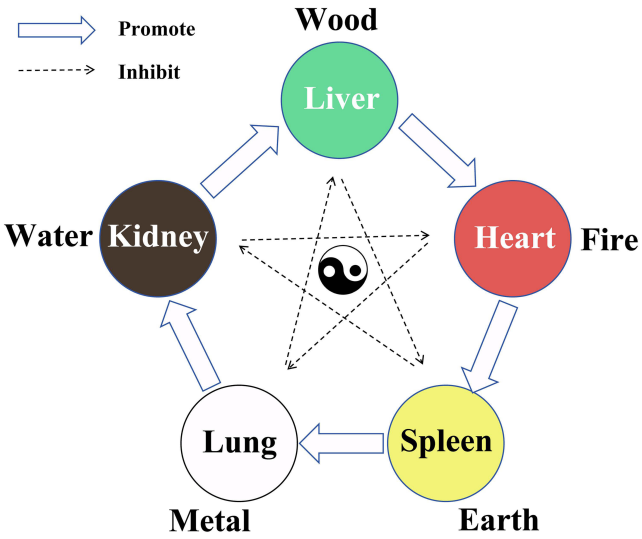

Figure 1 Five elements and five colors.

fortifying the dermal barrier. Employing TCM principles, herbs with blood-activating and stasis-dispelling properties that are contraindicated during pregnancy were excluded. Subsequently, a quintet of botanicals was selected, each relating to one of the five colors, culminating in a unique pentachromatic botanical composition, comprising mung bean, white truffle, black truffle, chrysanthemum, and Lycium barbarum.

In TCM, a formulary is composed of a diverse array of herbal ingredients, where certain herbs serve as the principal therapeutic agents, while others function as adjuncts or moderators to enhance the efficacy of the primary herbs. This strategy of formulating herbal combinations, underpinned by the TCM's empirical wisdom of the monarch-minister-assistant-envoy (jun-chen-zuo-shi) paradigm, mirrors the synergistic principles observed in contemporary medicine. However, the precise mechanisms by which these ingredients exert their therapeutic effects remain largely elusive. Unraveling these mechanisms through contemporary methodologies may support the rational and efficacious application of TCM. Consequently, a network pharmacology model has been constructed to investigate the constituent components and their underlying molecular mechanisms of action. Network pharmacology was performed in this study, and the aforementioned quintet of plants was analyzed, identifying 27 relevant bioactive constituents and projecting 636 potential targets. From this dataset, three primary targets were identified, including aldo-keto reductase 1B10 (AKR1B10), cyclin-dependent kinase 5 (CDK5), and cystathionine beta-synthase (CBS). Subsequent efficacy validation studies conducted at the cellular, tissue, organismal, and human levels have substantiated the significant anti-aging and reparative properties of this pentachromatic herbal combination.

Materials and Methods

Network Pharmacology

The components of mung bean, chrysanthemum, and Lycium barbarum were extracted from the TCMSP database (https://tcmsp-e.com/tcmsp.php), and the components of black and white truffles were obtained from published related documents.[20,21] The collected chemical components were predicted by the PreADMET Web server (https://preadmet.webservice.bmdrc.org/) to obtain the core components. Targets with predictive score (probability) greater than 0 were screened through the PubChem database and the Similarity ensemble approach (SEA) database, and the core component targets of five plants were sorted out. The core component-target (C-T) network was constructed by Cytoscape (3.10.1).

Reagents and Materials

Mouse embryo fibroblast 3T3 was purchased from Beyotime Biotechnology(China), and it was certified that the company was not contaminated by mycoplasma. Fetal bovine serum (FBS) and DMEM were purchased from Thermo Fisher Scientific biochemical products Co., Ltd. Penicillin and streptomycin (100X) and cell aging β-galactosidase staining kit were purchased from Beyotime Biotechnology, China. Culture medium for ex vivo skin models was purchased from Boxi Biotechnology (China). Vitamin C (VC) and Vitamin E (VE) were obtained from Sigma-Aldrich (USA). Phosphate-buffered saline (PBS) was purchased from Solarbio (China). Xylene was purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Masson's Trichrome Staining Kit was obtained from Eke Biotechnology (China). Hematoxylin and eosin staining reagents were purchased from Beyotime Biotechnology (China). Paraformaldehyde was obtained from Biosharp (China). Collagen IV antibody was purchased from Abcam (UK). The isolated skin used in this experiment comes from Guangdong Boxi Biotechnology Co., Ltd.

Equipment

The carbon dioxide incubator (Model 150I, Thermo Fisher Scientific, USA) was purchased from Thermo Fisher Scientific (USA). The laminar flow hood (Model SW-CJ-1F, Sujing Antai, China) was purchased from Sujing Antai (China). The UVA irradiation device, as well as the UVB irradiation device (Philips, Netherlands), were obtained from Philips (Netherlands). The upright microscope (Model BX53, Japan) was purchased from Olympus (Japan). The inverted fluorescence microscope (Model MF52-N) was purchased from Guangzhou Mingmei Optoelectronic Technology Co., Ltd.

Experiments

Cell Experiments

Sample Preparation

The test sample was diluted to a 4% (v/v) solution using pure water and filtered through a 0.22 μm membrane to obtain the working solution.

For the scratch assay, the working solution was used to prepare a basal medium containing the test sample. Controls included basal medium alone (negative control) and incomplete medium without serum (positive control).

For the cell viability and β-galactosidase staining assays, the test sample was either added alone to basal medium (for viability assessment) or added to basal medium containing hydrogen peroxide (H_2O_2) to induce cellular senescence.

Negative controls consisted of basal medium alone (for the cell viability assay) or basal medium with H_2O_2 (for the β-galactosidase staining assay).

Cell Viability Assay

Fibroblasts were seeded into 96-well plates and cultured for 24 hours. The culture medium was then replaced with basal medium containing various concentrations of the test sample.

For the β-galactosidase staining viability assessment, H_2O_2 was added to the medium as required.

After 24 hours of incubation, cell viability was assessed using the MTT assay, and absorbance was measured at 490 nm (OD_{490} nm) using a microplate reader.

Fibroblast Scratch Assay

3T3 fibroblasts were seeded into 6-well plates and cultured until reaching near confluency. Sterile pipette tips were used to create linear scratches across the cell monolayer. Detached cells were removed by washing with PBS.

The medium was then replaced with basal medium (negative control), incomplete medium (positive control), or medium containing the test sample.

Images of the scratch areas were captured at 0 hours and 24 hours using an MF52-N microscope to evaluate cell migration.

β-Galactosidase Staining Assay

Human dermal fibroblasts were seeded into 6-well plates and cultured for 24 hours. The cells were then fixed with β-galactosidase fixative solution at room temperature for 15 minutes.

Subsequently, the cells were incubated overnight at 37°C with β-galactosidase staining solution.

Blue-stained senescent cells were observed under a light microscope.

Data Analysis

Quantitative Image Analysis and Wound Closure Rate Calculation. Quantitative analysis of wound area images and β-galactosidase-stained images at 0 h and 24 h was performed using Image-Pro Plus (IPP) software.

The wound closure rate was calculated according to the following formula:

$$\text{Wound Closure Rate}(\%) = \left[1 - \frac{\text{Wound Area at 24h}}{\text{Wound Area at 0h}}\right] * 100$$

Composition and Enhancement Rate Calculation of the Five-Colored Plant Combination. The five-colored plant combination was composed of black truffle extract (35%), white truffle extract (35%), goji berry extract (10%), chrysanthemum extract (10%), and mung bean extract (10%).

To calculate the enhancement rate, the expected value was determined by multiplying the individual result of each extract at a 0.5% concentration by its proportional composition percentage, and then summing the weighted results.

The actual value was obtained by evaluating the combined formulation at a 0.5% concentration.

The enhancement rate was calculated using the following formula:

$$\text{Enhancement Rate}(\%) = \frac{\text{Actual Value} - \text{Expected Value}}{\text{Expected Value}} * 100$$

Ex vivo Skin Experiments

Culture of ex vivo Skin Model

The isolated skin (from Guangdong Boxi Biotechnology Co., Ltd.) used in this experiment has been approved by Guangdong Boxi Ethics Committee (batch number: GDLL2024011), and all work is carried out in accordance with relevant guidelines and regulations. Human ex vivo skin samples were trimmed into sections measuring approximately 24 ± 2 mm^2. Each sample was placed into a well of a 6-well plate containing 3.7 mL of culture medium. Samples were incubated at 37°C with 5% CO_2, and the medium was replaced daily.

UVA/UVB Irradiation and Drug Treatment

After 48 hours of culture, the samples were irradiated daily for four consecutive days with UVA (30 J/cm^2) and UVB (50 mJ/cm^2) using a Philips UVA and UVB irradiation system. Following each irradiation session, the medium was refreshed, and drug treatments were administered.

For treatment, 2μL of test formulation was applied directly to the skin surface. The control group received no irradiation or drug treatment, while the positive control group was irradiated but not treated with any formulation. After the four-day irradiation period, samples were further cultured for three days without further irradiation while still receiving daily drug treatment.

Tissue Processing

At the end of the treatment period, skin samples were rinsed gently with sterile PBS to remove residual reagents. Residual liquid was removed using sterile cotton swabs. For histological and collagen fiber analysis, samples were fixed with 4% paraformaldehyde for 24 hours. Thereafter, the samples were processed for hematoxylin and eosin (H&E) staining and Masson's trichrome staining.

Histological sections were observed and imaged under a light microscope for further analysis. For immunofluorescence detection, skin samples were similarly fixed with 4% paraformaldehyde for 24 hours. Immunofluorescence staining was performed to evaluate the expression levels of type IV collagen and other markers.

Enhancement Rate Calculation

The enhancement rate was calculated using the following formula:

$$\text{Enhancement Rate}\,(\%) = \frac{\text{Sample Group} - \text{Postive Control Group}}{\text{Postive Control Group}} * 100$$

Statistical Analysis

Statistical analysis was conducted using Excel (Microsoft Office, USA). Comparisons between groups were performed using a one-way ANOVA with post-hoc test. A P-value < 0.05 was considered statistically significant, and a P-value < 0.01 was considered highly significant.

Results

Network Pharmacology

A total of 587 components have been isolated from mung bean, chrysanthemum, and goji berry based on the TCMSP platform, while 399 components were identified from white and black truffle based on published literature. After optimization, 27 core components were finally obtained, and 636 relevant targets were predicted. A component-target (C-T) network was constructed using these 27 components and 636 targets (Figure 2). After literature research, three primary targets were identified, which were involved in skin anti-aging and repair. Notably, cystathionine-β-synthase (CBS) is an enzyme that produces hydrogen sulfide, and depletion of CBS is a rate-limiting enzyme in the reverse sulfur transfer pathway, which can induce premature aging of human endothelial cells.[22] Secondly, hydrogen sulfide is

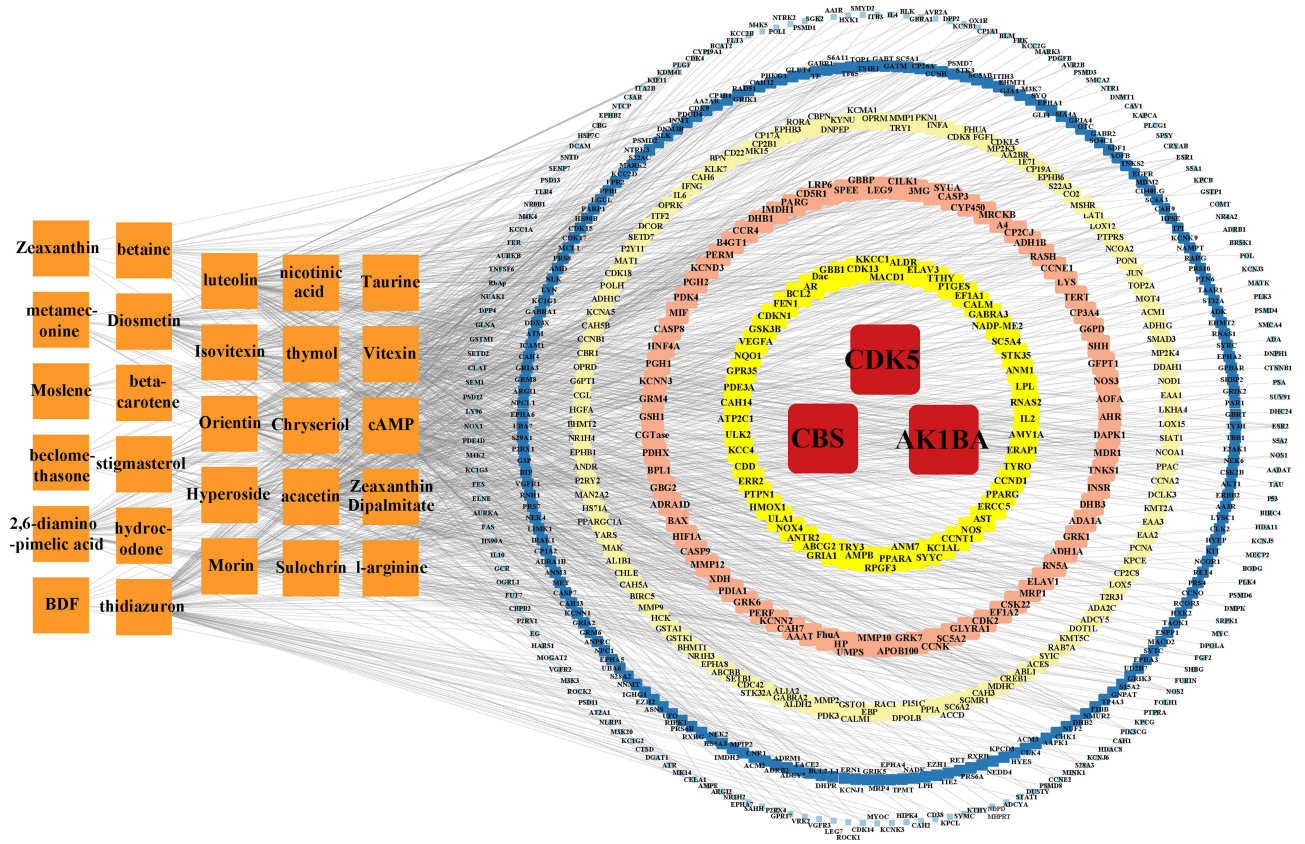

Figure 2 Component-target diagram.

considered an important endogenous gas transmitter. Studies have shown that H_2S may exert cell protection and anti-aging effects, and active sulfur substances support cell proliferation and regulate cell aging.[23,24] Some researchers have found that Cyclin-dependent kinase 5 (CDK5) is involved in the process of cell aging; knocking down or inhibiting CDK5 reduces the number of aging endothelial cells.[25–27] Aldo-keto reductase 1B10 (AKR1B10) target has also been found to be involved in the process of cell aging.[28] Secondly, all 636 targets were analyzed by KEGG enrichment, and 143 pathways were screened according to a $P<0.01$ threshold. Among the top 20 pathways, the AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, p53 signaling pathway, and estrogen signaling pathway were found to play a significant role in skin aging (Figure 3). Advanced glycation end products (AGEs) promote collagen cross-linking and inflammation through the RAGE receptor, resulting in a decrease in skin elasticity.[29] Some researchers have found that inhibiting PI3K-Akt overactivation can reduce oxidative damage and promote autophagy to delay skin aging.[30] Furthermore, p53 plays an important role in the aging of organisms. Kim et al found that activating p53 can accelerate the loss of subcutaneous fat, reduce the activity of sebaceous glands, and lead to skin aging and dryness.[31] Moreover, estrogen can maintain skin elasticity by promoting collagen synthesis and inhibiting MMPs, and the decline of estrogen after menopause accelerates skin aging.[32–34] Therefore, the selected five plants may play a major role in skin aging.

Cell Experiments

Fibroblast Scratch Assay

After 24 h, the positive control group demonstrated effective fibroblast scratch healing, achieving a healing rate of 66.48%. In the negative control group, a lower degree of scar healing was observed, with a healing rate of 10.43%. At the 0.5% concentration of white truffle extract, black truffle extract, Lycium barbarum extract, chrysanthemum extract, and mung bean extract, the wound healing rates of fibroblasts after 24 h were 57.59%, 44.12%, 42.85%, 41.71%, and

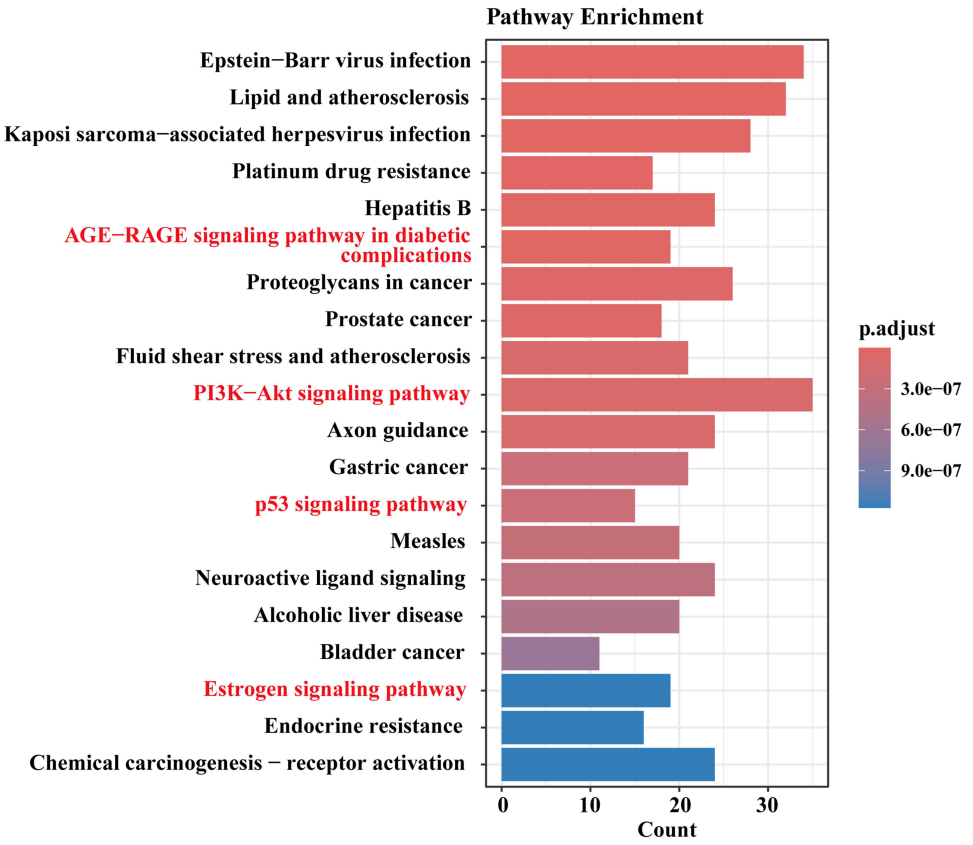

Figure 3 KEGG enrichment analysis of all targets of the five-colored plant combination. The bold red font represents four pathways related to skin aging.

47.04%, respectively. Using the 0.5% concentration of the combined plants, the wound healing rate of fibroblasts reached 59.89%, which was 22.83% higher than that of the individual components (Figure 4).

Cellular Aging Assay

In the negative control group, the proportion of blue area in β-galactosidase staining of human fibroblasts was 2.319%. At the 0.5% concentration, the blue area in β-galactosidase staining of human fibroblasts in the experimental group corresponding to white truffle extract, black truffle extract, Lycium barbarum extract, chrysanthemum extract, and mung bean extract was 1.863%, 1.196%, 1.468%, 1.306%, and 0.929%, respectively. These results were 19.69% lower compared to the negative control. The five-colored plant combination at 0.5% concentration resulted in a blue area of 0.777%, which was 76.32% lower than that of the negative control and 101.53% higher than that of the individual raw materials (Figure 5).

Ex vivo Skin Experiments

In the ex vivo skin model, the thickness of the epidermis living cell layer, collagen fiber content, and collagen IV content of the COM group all increased significantly compared with the control group, achieving increases of 28.16%, 49.12%, and 100.00%, respectively. In addition, the epidermis thickness increased significantly, and the ridge structure of DEJ was more obvious, indicating that the five-colored plant combination can improve the tissue morphology, collagen fiber content, and collagen IV content (Figure 6).

Discussion and Conclusion

Network pharmacology has been widely adopted in previous studies due to its compatibility with the holistic principles of Traditional Chinese Medicine. This methodology has been utilized to investigate the systematic mechanisms involving multiple components, targets, and pathways. Moreover, its predictive power in uncovering the mechanistic basis of plant-

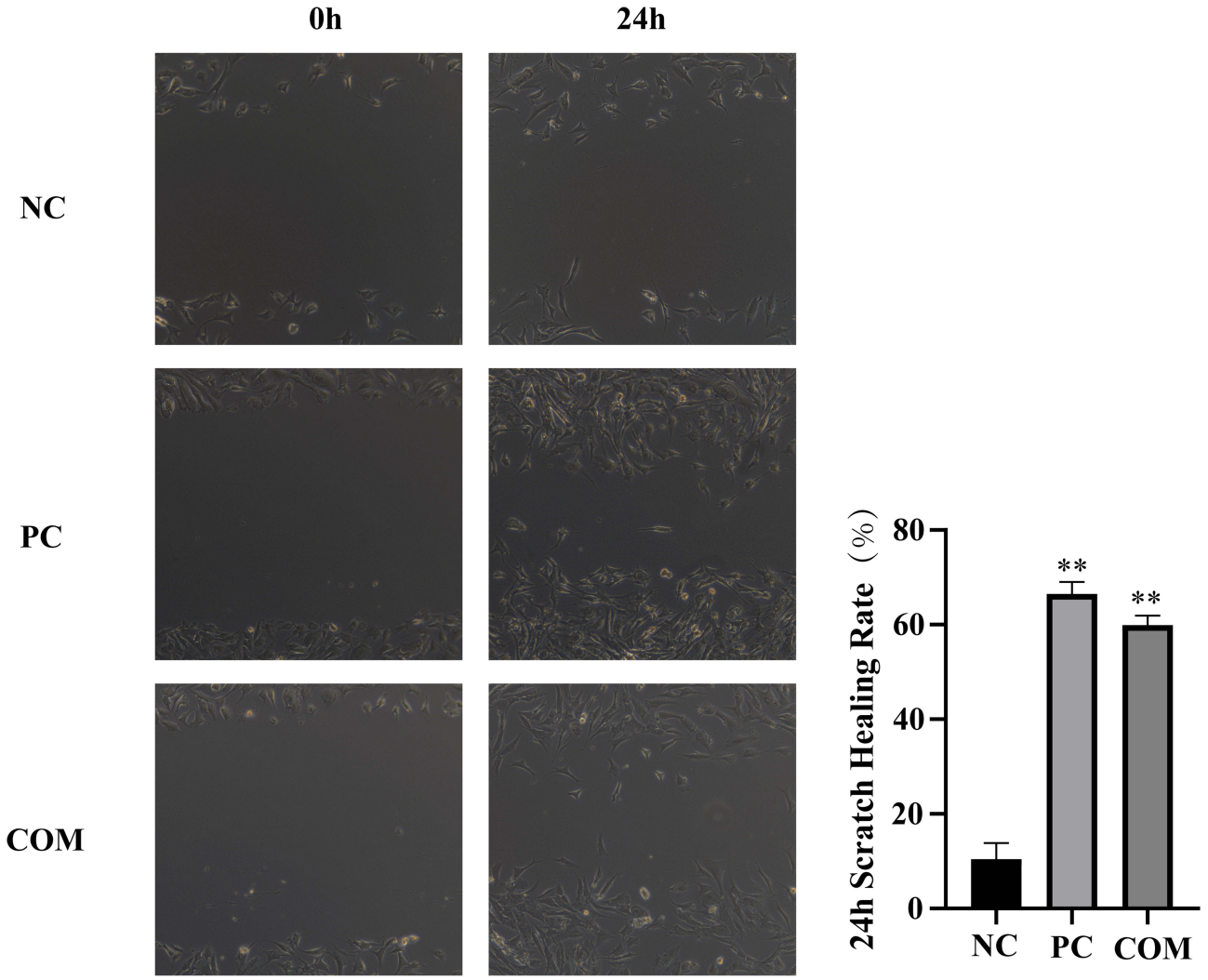

Figure 4 Experimental results of the fibroblast scratch assay. NC represents the negative control, PC represents the positive control, and COM indicates the five-colored plant combination. After 24 h, most of the scratches in the PC group were healed, achieving a healing rate of 66.48%. In contrast, a lower wound healing was observed in the NC group, reaching a healing rate of only 10.43%. The 0.5% COM group showed a high cell activity, and the wound healing rate of fibroblasts was 59.89% after 24 hours, demonstrating effective wound healing.Compared with the NC group, the $P < 0.01$ was represented by **.

based formulations has been validated by empirical research.[35–37] The Huangdi Neijing is one of the earliest and most authoritative medical texts in China, serving as a foundational canon of Traditional Chinese Medicine (TCM). This text has been translated into multiple languages, contributing to the cross-cultural transmission of TCM knowledge. Among its core theoretical frameworks, the doctrine of the Five Organs (Wu Zang) has drawn sustained scholarly attention and remains a central topic in contemporary TCM research.[38–41] This study is the first to integrate the traditional theory of "the Five Colors Entering the Five Organs", as documented in the Huangdi Neijing, into a modern cosmetic context. A specific combination of five-colored plants was constructed based on this theory, and network pharmacology was utilized to systematically explore their potential mechanisms in skin aging intervention.

Studies have revealed the association between traditional Chinese medicine and aging, but have primarily focused on functional foods and drugs, such as the effect of feeding Bazhen soup on skin photoaging.[42,43] However, studies related to cosmetics and skin remain scarce. Previous cosmetic studies have explored the efficacy of botanical ingredients with anti-aging effects, but no research has integrated the theoretical doctrines of Chinese medicine.[13,44–46] The present study is the first to integrate five-colored botanical ingredients based on the Traditional Chinese Medicine (TCM) theory of "holistic synergy", using color classification as a key principle for plant combination. It also represents the first attempt to directly associate a classical TCM theory with functional cosmetic outcomes through experimental validation. The results

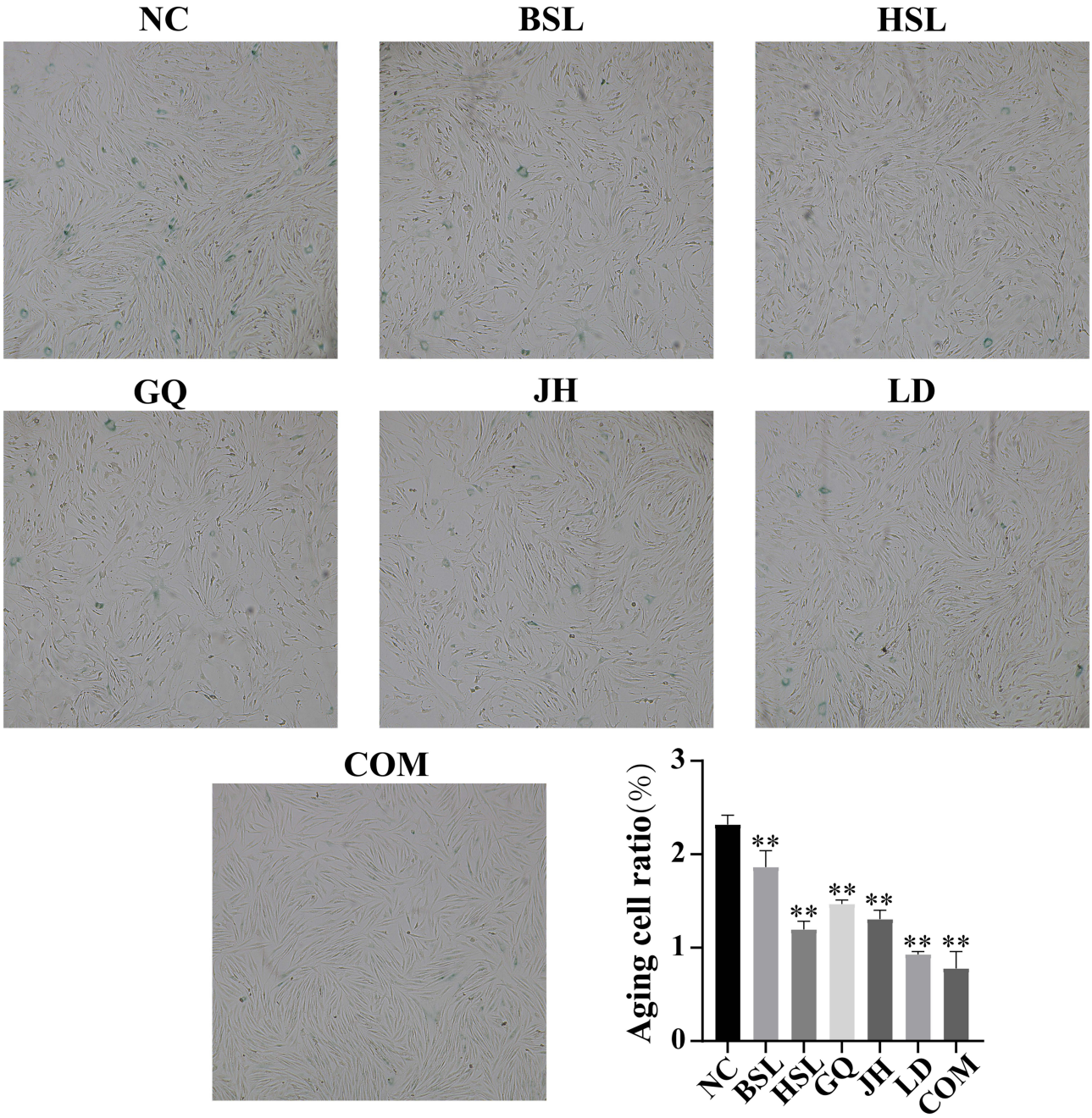

Figure 5 Experimental results of cell anti-aging. NC is the negative control, and BSL, HSL, GQ, JH, LD, and COM represent the white truffle, black truffle, Lycium barbarum, chrysanthemum, mung bean, and five-colored plant combination. Compared with the NC group, the blue areas of BSL, HSL, GQ, JH, LD, and COM decreased by 19.66%, 48.43%, 36.71%, 43.70%, 59.96%, and 76.32%, respectively. The five-colored plant combination showed a 101.53% higher blue area compared with the individual components. Compared with the NC group, the $P < 0.01$ was represented by **.

demonstrated that the specific combination of five-colored plants produces a significantly greater synergistic effect in anti-aging and skin repair compared to individual ingredients. This finding not only supports the traditional theory of "Five Colors Entering the Five Organs" but also highlights its relevance in modern scientific contexts. Therefore, this study contributes to the modernization of classical Chinese medicine by transforming abstract theory into a testable, efficacious strategy for skin anti-aging. The study also addresses a critical research gap by establishing a comprehensive framework linking TCM theory, plant-based formulation, and cosmetic efficacy evaluation.

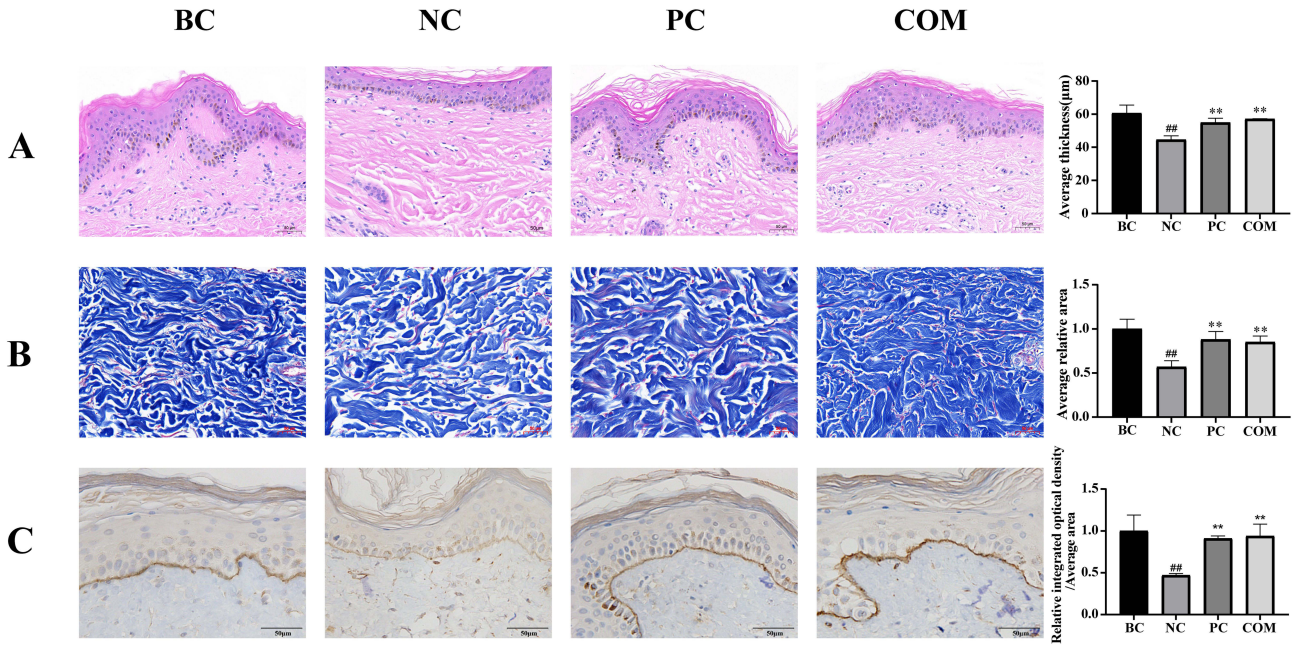

Figure 6 Ex vivo Skin Experiment Results. BC represents the blank control, NC represents the negative control (UVA+UVB), PC indicates the positive control (100μg/mL VC+7μg/mL VE+UVA+UVB), and COM denotes the five-colored plant combination. (**A**) Morphological staining of epidermal living cell layer revealed a significantly higher thickness of living cell layer in the COM group compared with NC group, showing an increase of 28.16%; (**B**) Following collagen fiber staining, the COM group demonstrated a significant increase of 49.12% in the content of collagen fiber compared with the NC group. (**C**) The immunohistochemical results of collagen IV showed a significant increase of 100.00% in the content of collagen IV in the COM group compared with the NC group. In the figure, ## indicates a significance of $P < 0.01$ compared with the BC group; ** indicates a significance of $P < 0.01$ compared with the NC group.

Therefore, the targets and pathways related to the five-colored plant combination were analyzed by network pharmacology to explore their specific role in skin repair and aging delay. The literature search showed that many targets were involved in skin anti-aging and repair (Figure 2). Additionally, KEGG pathway enrichment revealed modulation of the AGE-RAGE signaling pathway in diabetic complications, PI3K-Akt signaling pathway, p53 signaling pathway, and estrogen signaling pathway, thereby regulating skin collagen and skin aging (Figure 3). Studies[47–49] have shown that advanced glycation end products (AGEs) accumulate in the skin with age and high glucose status, which activate downstream signals such as NADPH oxidase, ERK1/2, and MAPK after binding to their receptor RAGE. This induces oxidative stress and inflammatory reaction, accelerates the functional decline of dermal cells (especially fibroblasts), promotes the glycosylation of collagen (especially types I and IV), and hardens the structure and increases cross-linking. Activation of NF-κB induces apoptosis and inhibits normal cell growth, which leads to the decline of migration and repair ability. Zhang[50] and Mercurio[51] reported that PI3K/Akt inhibits apoptosis and delays aging by regulating p53 and FoxO. PI3K/AKT can also affect the synthesis of type I collagen, maintain the structure of DEJ, maintain the thickness of epidermis, promote the migration of keratinocytes and dermal cells, and enhance wound repair.[52–57] PI3K/Akt is an important signal pathway for regulating cell proliferation, survival, migration, fibroblast activity, collagen synthesis, and wound healing in the skin. The P53 signaling pathway acts as an "aging sensor" in skin, which inhibits the PI3K-Akt pathway, promotes p21 and cell cycle arrest, and induces the aging of skin fibroblasts and keratinocytes when activated.[58–60] High expression of DNA damage induced by ultraviolet rays plays an essential role in skin photoaging.[61] Moreover, the estrogen signaling pathway regulates various skin functions through its receptors (Erα and ERβ), stimulates the synthesis of type IV and type I collagen, increases the thickness of dermis,[62] promotes fibroblast migration and skin repair, regulates antioxidant and anti-apoptosis functions, and delays the aging of skin cells.[33,63,64] These findings support the effects of the five-colored plant combination in delaying aging. However, the specific mechanisms of these pathways still need to be verified by in vivo and in vitro experiments.

Finally, the effect of the five-colored plant combination was demonstrated by wound healing assays, β-galactosidase staining, and observing the changes in epidermis thickness and collagen in vitro. The experimental results revealed that

the formula can significantly promote the wound healing of fibroblasts. Compared with the individual raw materials, the five-colored plant combination achieved a 22.83% higher healing rate, and a significantly lower blue area in the β-galactosidase staining. Compared with individual raw materials, the plant combination showed a 101.53% increase in blue area, indicating a significant inhibition of cell aging. Compared with the control group, the five-colored plant combination increased the epidermis thickness, collagen fiber content, and type IV collagen content by 28.16%, 49.12%, and 100.00%, respectively. The tissue section staining revealed that the five-colored plant combination improved the skin tissue morphology and confirmed its ability to delay skin aging.

In summary, this study systematically investigated the potential mechanisms of a five-colored plant combination in mitigating skin aging, integrating network pharmacology with in vitro experimental validation. The present study innovatively bridges the five-color theory from the Huangdi Neijing with modern anti-aging research, and establishes a research framework to confirm the synergistic potential of chromatically diverse botanical ingredients. These findings provide empirical support for the modernization of traditional Chinese medical theories. Nevertheless, the limitations of this study should be acknowledged, including the lack of detailed analysis on the specific bioactive components and the precise molecular mechanisms underlying the synergistic effects of the five-colored plant combination.

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Disclosure

The authors declare no conflicts of interest.

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