Development and Efficacy Evaluation of a Sanbai-Based Compound Essence Optimized by Response Surface Methodology

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Abstract

Using Angelica dahurica, Bletilla striata, and Poria cocos extracts as the core ingredients, the compound herbal skin care serum was prepared and tested for its optimal ratio and performance. The sensory comprehensive evaluation method and response surface test were used to optimize the formulation of the herbal compound serum; its performance in antioxidant and whitening was comprehensively evaluated by the DPPH free radical scavenging test and tyrosinase activity inhibition test. The optimal ratios of the herbal serum were 6.18% for the compound herbal extract, 8.82% for glycerol, and 0.13% for xanthan gum. The texture of the compound herbal extract prepared by the optimal process was fine and stable, and it was preliminarily judged to have good antioxidant efficacy. The compound herbal essence prepared in this study has good properties and can provide a reference basis for the development and utilisation of herbal skin care products.

Keywords

Antioxidant Activity; Response Surface Methodology Optimization; Skincare Product Development.

1. Introduction

Sanbai San is a renowned formula from the Song Dynasty's "Taiping Shenghui Fang," composed of Angelica dahurica, Bletilla striata, and Poria cocos. The combination of these three herbs aligns with the theory that "the lung governs the skin and hair," collectively achieving the effects of promoting lung function, moisturizing the skin, dispersing blood stasis, and eliminating spots[1]. The "Compendium of Materia Medica" records that Angelica dahurica "nourishes the skin and enhances complexion," Bletilla striata "cleanses the face of darkness and removes spots," and Poria cocos "promotes the production of body fluids and regulates water metabolism"[2]. These descriptions reflect the traditional Chinese medicine principle of "nourishing whiteness with white" to improve skin tone[3], demonstrating its potential applications in skin whitening, antioxidant protection, and skin barrier repair.

Pigmentation is a disfiguring skin condition caused by melanin metabolism disorders, significantly impacting patients' psychological well-being and social activities[4]. Its core pathological mechanisms involve abnormal elevation of tyrosinase activity leading to excessive melanin synthesis[5], as well as UV-induced accumulation of reactive oxygen species (ROS) causing oxidative skin damage[6]. Given these mechanisms, skin whitening must address both tyrosinase activity inhibition and free radical scavenging. Traditional Chinese medicine has a long history of application in beauty care[7] and has accumulated a solid theoretical foundation and practical experience in skin care[8]. Its theoretical core of "holistic view" and "differential diagnosis and treatment" provides a unique approach for developing natural, safe, and effective skin whitening products[9]. Previous studies on the application of such classic formulas in modern skincare product development and application remain insufficient.

Based on this, this study uses Sanbai San as the theoretical foundation, applying the compound extract to the construction of an essence system and using response surface methodology to optimize key ratios. Additionally, using DPPH free radical scavenging rate and tyrosinase activity inhibition rate as core indicators, the study verifies the product's in vitro antioxidant and skin-whitening efficacy, providing new experimental evidence and insights for the development of TCM skincare products.

2. Materials and Instruments

2.1. Materials

Angelica dahurica, Bletilla striata, Poria cocos (Hebei Hehuachi Pharmaceutical Co., Ltd.); Glycerin (Guangzhou Panyu Liqianghua Factory); Allantoin (Guangzhou Mei Yi Biotechnology Co., Ltd.); Butylene glycol (Guangzhou Qixu Chemical Factory); Methylparaben (Shandong Yuezhong Food Co., Ltd.); Sodium hyaluronate (Shaanxi Xingda Biotechnology Co., Ltd.); L-DOPA (Shanghai Shifeng Biotechnology Co., Ltd.); Tyrosinase (Guangzhou Huilong Biotechnology Co., Ltd.); All other reagents are analytical grade.

2.2. Instruments

Magnetic Stirring Constant Temperature Water Bath (Changzhou Ronghua Instrument Manufacturing Co., Ltd.); T6 New Century UV-Vis Spectrophotometer (Beijing Puxi General Instrument Co., Ltd.).

3. Preparation of Essence Products

3.1. Preparation of Traditional Chinese Medicine Extracts

Take Angelica dahurica, Bletilla striata, and Poria cocos as medicinal materials. Dry them at a constant temperature of 60°C, then grind them through an 80-mesh sieve. Precisely weigh 10 g of each powdered herb, add distilled water at a ratio of 1:40, and perform ultrasonic extraction at 70°C and 250 W for 80 minutes. Filter the mixture, then reduce the filtrate under vacuum to one-fifth of its original volume. Store the solution at 4°C in a light-protected environment.

3.2. Preparation of Essence Products

The essence described in this study employs a three-phase system, with the components and their mass percentage ranges as follows:

1.Phase A. Contains glycerin (3%-15%), 1,3-butanediol (3%), sodium hyaluronate (0.3%), xanthan gum (0.05%-0.25%), tocopheryl acetate (0.5%), and methylparaben (0.2%).

2.Phase B. Contains betaine (2%), natural citrus essential oil (0.6%), and distilled water (to make up the remainder to 100%).

3. Phase C. Contains a compound extract of traditional Chinese medicinal herbs (2%-10%), which serves as the core active ingredient in this study.

3.3. Single-Factor Experiment

The essence described in this study employs a three-phase system, with the components and their mass percentage ranges as follows:

Using sensory evaluation methods as outlined in Section 2.2, while keeping other ingredient ratios constant, the following factors were examined: glycerin addition (3%, 6%, 9%, 12%, 15%) and xanthan gum addition (0.05%, 0.1%, 0.15%, 0.2%, 0.25%) on sensory scores[10]. When studying one factor, the remaining three factors were set to their midpoints.

Ten evaluators with experience in cosmetic sensory evaluation (five males and five females) scored samples from the single-factor experiments[11]. The sensory evaluation criteria are detailed in Table I. Scores were recorded on a 100-point scale, rounded to one decimal place, with the final score representing the average of all individual evaluations.

Table I. Sensory Evaluation Criteria

Evaluation Indicators	Rating Criteria	Rating Range
Appearance (15 points)	Color is uniform and translucent	11～15
	Minor color variation or slight cloudiness	6～10
	Noticeable separation, sedimentation, or abnormal coloration	0～5
Odor (20 points)	No pungent or cheap fragrance odor	14～20
	Slightly strong aroma, but no discomfort	7～13
	Pungent off-odors or pronounced fragrance odor	0～6
Extensibility (15 points)	Easy to apply, no pulling sensation, smooth texture	11～15
	Easily spreads with minimal resistance	6～10
	Difficult to apply, may clump or feel sticky	0～5
Moisturizing Properties (20 points)	Skin feels hydrated and plump, without stickiness or dryness.	14～20
	The moisturizing effect is short-lived, with slight stickiness or mild dryness.	7～13
	Skin feels noticeably sticky or dry with flaking.	0～6
Efficacy (30 points)	Significant skin brightening-effect	21～30
	Slight but not noticeable skin brightening effect	11～20
	No noticeable efficacy demonstrated	0～10

3.4. Response Surface Optimization

Based on single-factor experimental results, a three-factor, three-level response surface experiment was designed using Design-Expert 13 software. The factors were compound extract addition amount (A), glycerol addition amount (B), and xanthan gum addition amount (C), with sensory score (Y) as the response value. This determined the optimal formulation ratio [12]. The factors and levels are shown in Table II..

Table II. Factors And Levels of Box-Behnken Experimental Design

level	factor		
	A	B	C
-1	6	6	0.1
0	8	9	0.15
1	10	12	0.2

3.5. In Vitro Experiments

The essence described in this study employs a three-phase system, with the components and their mass percentage ranges as follows:

DPPH RADICAL SCAVENGING ASSAY

Precisely weigh the optimal formulation essence product and vitamin C reference substance. Dilute with anhydrous ethanol to prepare test solutions at concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL. Take 1.0 mL of each test solution and add 3.0 mL of 0.2 mmol/L DPPH ethanol solution. Mix thoroughly by vortexing. Use 1.0 mL of anhydrous ethanol as a blank control. Incubate all samples at room temperature, protected from light, for 30 minutes. Measure the absorbance at 517 nm [13], performing three parallel measurements. The free radical scavenging rate was calculated using Formula (1), and the dose-response curve was plotted using Origin 2025 software.

$$\text{Clearance rate} = (1 - A/A0)\times 100\% \quad (1)$$

IN VITRO TYROSINASE ACTIVITY INHIBITION ASSAY

Precisely weighed samples of the optimized formulation were prepared into a series of test solutions at concentrations of 0.05, 0.1, 0.2, 0.4, and 0.8 mg/mL using a gradient dilution method with anhydrous ethanol. The reaction system was constructed as shown in TABLE III. Measurements for A1 and A2 were performed once, while A3 and A4 were measured in triplicate. Calculate the tyrosinase inhibition rate using Formula (2) and establish the dose-response relationship curve using Origin 2025 software [14].

$$IR(\%)=\frac{(A1-A2)-(A3-A4)}{A1-A2}\times 100\% \quad (2)$$

Table III. Experimental Reaction System Design for Tyrosinase Activity Inhibition Experiment

solvent	Additional amount/mL			
	A1	A2	A3	A4
Sample	0	0	1	1
Tyrosinase solution	1	0	1	0
Phosphate buffer solution	2	3	1	2
L-DOPA solution	1	1	1	1

4. Results

4.1. Single-Factor Experimental Design and Results

Sensory evaluation results under different component addition levels are plotted in Figure 1, revealing that the parameters of each component addition significantly influence product quality. Optimal sensory scores were achieved when the compound extract addition ranged from 6% to 10%, glycerin addition from 6% to 12%, and xanthan gum addition from 0.1% to 0.2%. Based on these findings, response surface experiments were conducted to further optimize the formulation ratios for the essence product.

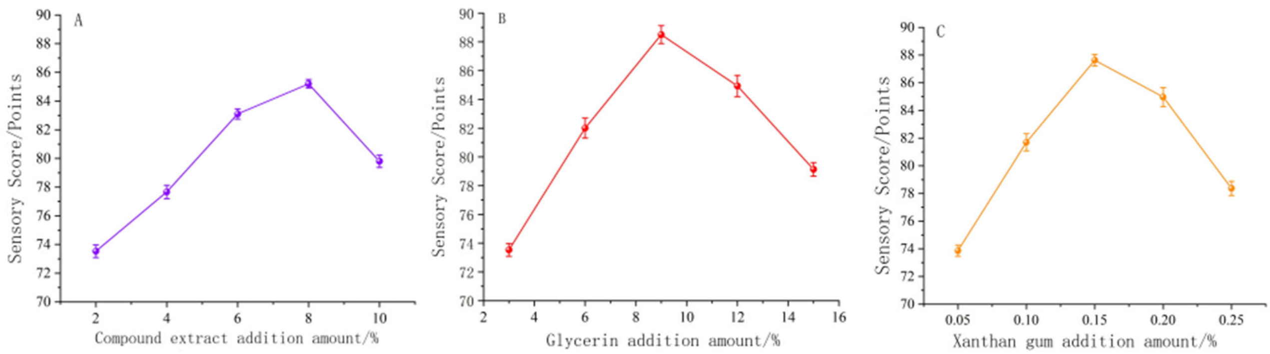

Figure 1. Effect of single factors on the sensory evaluation of essence.

4.2. Response Surface Analysis Results

Based on single-factor experimental results, a three-factor, three-level response surface experiment was designed with compound extract addition amount (A), glycerol addition amount (B), and xanthan gum addition amount (C) as factors, and sensory score (Y) as the response value. The experimental design and results are shown in Table IV, and the analysis of variance results are presented in Table V.

Table IV. Response Surface Experiment Results

Number	A	B	C	Sensory Score (Y/Points)
1	-1	-1	0	83.3
2	1	-1	0	75.6
3	-1	1	0	82.4
4	1	1	0	77.8
5	-1	0	-1	84.2
6	1	0	-1	76.5
7	-1	0	1	83.8
8	1	0	1	77.8
9	0	-1	-1	85.6
10	0	1	-1	85.3
11	0	-1	1	85.4
12	0	1	1	84.8
13	0	0	0	90.7
14	0	0	0	90.3
15	0	0	0	90.9
16	0	0	0	91
17	0	0	0	90.5

Table V. Anova of The Regression Model

Sources of variance	Degree of freedom	Sum of Squares	Mean Square	F-value	P-value	Significance
Model	431.94	9	47.99	224.27	<0.0001	**
A	84.5	1	84.5	394.86	<0.0001	**
B	0.02	1	0.02	0.0935	0.7687	
C	0.005	1	0.005	0.0234	0.8828	
AB	2.4	1	2.4	11.23	0.0122	**
AC	0.7225	1	0.7225	3.38	0.1088	
BC	0.0225	1	0.0225	0.1051	0.7552	
A^2	256.33	1	256.33	1197.82	<0.0001	**
B^2	40.53	1	40.53	189.39	<0.0001	**
C^2	22.32	1	22.32	104.31	<0.0001	**
Residual	1.5	7	0.214			
Lack of Fit	1.17	3	0.39	4.76	0.083	
Pure Error	0.328	4	0.082			
Cor Total	433.44	16				

Note: "*" indicates significant difference (P<0.05); "**" indicates extremely significant difference (P<0.01).

After data regression analysis, the fitted equation is $Y=90.68-3.25A+0.05B+0.025C+0.775AB+0.425AC-0.075BC-0.78A^2-3.1B^2-2.3C^2$. As shown in Table 4, when sensory scores serve as the response variable, the regression model is highly significant (P<0.01), while the misfit term is insignificant (P>0.05), indicating the model possesses high reliability [15]. The coefficient of determination R^2 is 0.9965, the adjusted coefficient of determination R^2adj is 0.9921, and the predicted coefficient of determination R^2Pre is 0.9556. These values indicate that the fitted regression equation exhibits ideal fitting quality and reliability [16], making the model suitable for analyzing and predicting the optimal formulation of the essence. Based on the P-values, factors A, A2, B2, and C2 exerted extremely significant effects on sensory scores (P<0.01), while factor AB had significant effects (P<0.05). According to the F-value, the influence of factors on sensory scores, from strongest to weakest, was A>B>C.

4.3. Response Surface Analysis Results

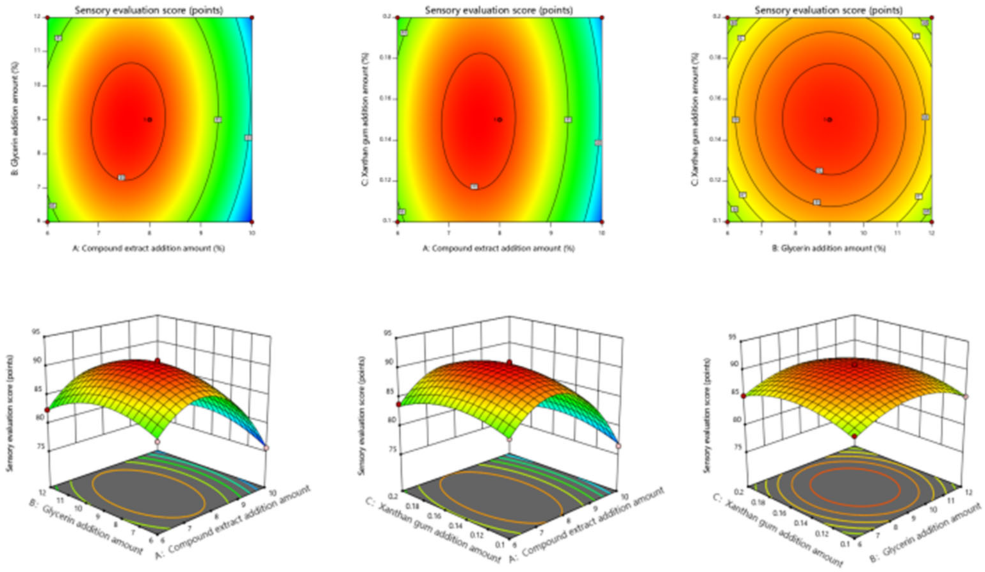

Figure 2. Three-dimensional star response surface and contour plot of each factor.

The effects of interactions among factors can be reflected by the steepness of response surface gradient changes [17]. As shown in Figure 3, the three-dimensional surface plot exhibiting the steepest gradient corresponds to the compound extract addition rate and glycerol addition rate, with contour lines forming distinct elliptical shapes, indicating significant interaction. With the maximum composite score as the optimization objective, the response optimizer determined the optimal raw material ratios: 7.523% compound herbal extract, 8.915% glycerin, and 0.152% xanthan gum. This formulation achieved a predicted sensory score of 91.0 points.

4.4. Results of the DPPH Radical Scavenging Assay

As shown in Figure 3, the DPPH radical scavenging activity of the essence product exhibits concentration dependence. Within the concentration range of 0.2–1.0 mg/mL, the scavenging rate significantly increases with rising product concentration. Although the essence product's scavenging activity against DPPH radicals is generally weaker than that of vitamin C, it still demonstrates strong scavenging activity, indicating its antioxidant potential.

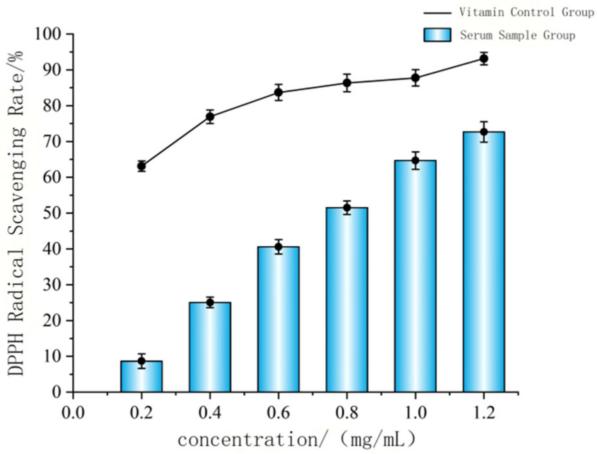

Figure 3. Scavenging effect on DPPH free radical

4.5. Results of Tyrosinase Activity Inhibition Assay

As shown in Figure 4, the serum product exhibits a clear dose-response relationship in inhibiting tyrosinase activity within the concentration range of 0.05 mg/mL to 0.2 mg/mL. As the sample mass concentration increases, its inhibitory effect strengthens, though it remains weaker than that of arbutin. When the concentration reaches 0.2%, the inhibitory effect levels off, and the dose-response relationship diminishes. Based on this tyrosinase activity inhibition, the results validate the whitening potential of this product.

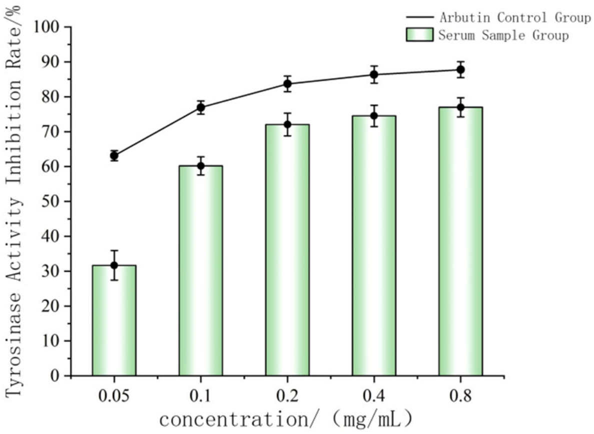

Figure 4. Tyrosinase activity inhibition results

5. Conclusion

This study utilized the compound herbal extract from the classical Chinese medicine formula Sanbai Powder to construct a serum formulation. Through sensory evaluation as the core response metric, combined with single-factor experiments and response surface design, the optimal formulation ratio was determined as 6.18% compound herbal extract, 8.82% glycerin, and 0.13% xanthan gum. The serum prepared using this formulation exhibits uniform texture, excellent sensory evaluation, and stable physicochemical properties.

The efficacy of this serum is theoretically grounded in the synergistic mechanisms of its three herbal components. Modern pharmacological research indicates that the coumarin compounds abundant in Angelica dahurica exhibit significant tyrosinase inhibitory activity[18], directly promoting melanin degradation[19]. The polysaccharides and benzyl compounds in Bletilla striata possess tyrosinase inhibitory capabilities[20], while its mucilage components demonstrate excellent film-forming and moisturizing properties[21]. This biological film not only reduces transepidermal water loss and promotes wound healing[22], but also exerts anti-inflammatory and antioxidant effects[23]. It further facilitates keratin metabolism, accelerating the shedding of aged keratinocytes containing melanin[24]. Triterpenoid compounds in Poria cocos have demonstrated potent antioxidant capacity, effectively scavenging free radicals and protecting skin cells from oxidative damage[25]. Its polysaccharide components exhibit superior moisturizing properties and promote skin barrier repair. The combination of these three herbs possesses a theoretical foundation and application potential for synergistic enhancement in whitening, antioxidant effects, and skin barrier repair.

In vitro experiments confirm this essence possesses significant antioxidant and whitening potential. DPPH radical scavenging assays demonstrate concentration-dependent antioxidant activity, with DPPH radical scavenging rates increasing with sample concentration[26]. Tyrosinase activity inhibition tests revealed an inhibition rate of 76.70% at a concentration of 0.8 mg/mL, suggesting its core active ingredients may act on the allosteric site of tyrosinase. This alters the enzyme conformation and reduces its affinity for the substrate L-DOPA, thereby effectively interfering with the melanin synthesis pathway[27]. This result corroborates the efficacy of the Sanbai Powder formula in promoting lung function, moisturizing skin, resolving stasis, and eliminating spots from a modern pharmacological perspective.

In summary, this study optimized the key ratios of the compound essence based on the classical formula Sanbai Powder using response surface methodology. The resulting essence product exhibits excellent sensory properties and stability, with in vitro efficacy tests confirming its significant antioxidant capacity and whitening activity. The development process fully integrates traditional Chinese medicine theory with modern formulation technology, validating the scientific basis of the “nourishing whiteness with whiteness” theory. It provides new insights and experimental evidence for developing herbal skin-whitening products aligned with the “natural, green, and healthy” consumer trend. This study offers theoretical foundations and technical support for creating herbal skincare products that combine traditional efficacy with modern formulation advantages.

The manuscript should include a conclusion. In this section, summarize what was described in your paper. Future directions may also be included in this section. Authors are strongly encouraged not to reference multiple figures or tables in the conclusion; these should be referenced in the body of the paper.

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