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"Explore the optimal solution for skin color: nicotinamide combined with traditional plant active essence to create a good complexion in all dimensions"

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1. Introduction

In today's era of pursuing beauty, whitening cosmetics have attracted much attention. It can not only satisfy people's yearning for fair skin, but also improve problems such as uneven skin tone and dullness, and enhance confidence. The main pigments in the skin are carotene, heme and melanin, among which melanin determines the depth of the skin. Melanin is produced within melanocytes[1]. The synthetic pathway is that tyrosine is first converted into dopa under the action of tyrosinase, and then dopaquinone is generated. Dopaquinone is then synthesized into melanin and eumelanin through different pathways[2]. Nicotinamide, as the most widely used chemical whitening agent, has an inhibitory effect of 35% to 68% on the melanin transport system and can also accelerate the renewal rate of keratinocytes, thereby accelerating melanin metabolism[3, 4]. Traditional Chinese medicine ingredients also play an important role in the field of whitening. For instance, the flavonoids contained in the glycyrrhiza uralensis (licorice) root extract can inhibit the activity of tyrosinase, interfere with the function of melanocytes and have antioxidant effects. Meanwhile, the glycyrrhizic acid in the glycyrrhiza uralensis (licorice) root extract can promote melanin metabolism and has a significant anti-inflammatory effect[5]. The components such as baicalein and baicalein in the scutellaria baicalensis root extract can inhibit the activity of tyrosinase, and also have antioxidant and anti-inflammatory effects[6]. The components in morus alba bark extract, such as morus alba bark extract D and mulberry flavonoids G, can effectively inhibit the activity of tyrosinase, reduce melanin production, and also have antioxidant, anti-inflammatory and melanin metabolism-promoting effect[7]. Lycium chinense fruit extract and artemisia capillaris extract both have the effects of whitening, antioxidation and anti-inflammation[8, 9].

2. Materials and Methods

The best combination of active substances was screened by in vitro experiments. Sample 1 was 2% niacinamide + 2% phytocompound solution (1:1). Sample 2 was 3% niacinamide + 1% phytocompound solution (3:1). Sample 3 was 3.6% niacinamide + 0.4% phytocompound solution (9:1). The phytocompound solution contained glycyrrhiza uralensis (licorice) root extract, scutellaria baicalensis root extract, morus alba bark extract, lycium chinense fruit extract and artemisia capillaris extract. Firstly, the tyrosinase inhibition rate, DPPH free radical scavenging rate and hyaluronidase inhibition rate were determined to select the combination of whitening, a00ntioxidant and anti-inflammatory effects. Furthermore, the tyrosinase activity and melanin content in B16-F10 cells were determined. Finally, the best combination was prepared into essence, which was tested on UV-induced human skin blackening model and volunteers' face to explore its whitening and fmacerating effect.

2.1 Materials and Equipment

Tyrosinase、 DPPH、L-tyrosine/L-dopa、B16-F10 mouse skin melanoma cells、DMEM High Glucose Medium、0.25% trypsin、VisioScan VC20 Plus (Courage&Khazaka, Germany)

2.2 In vitro evaluation methods

2.2.1 Inhibition of tyrosinase activity[10]

Tyrosinase dopa rate oxidation method was used to determine tyrosinase activity in vitro. Refer to Table 1. for the substances and addition amounts of tubes A, B, C and D. Add PBS (pH=6.8, 25 mmol/L), test solution and 0.05% L-dopa solution in sequence, Keep the temperature in a 37℃ water bath for 10 minutes, at the same time, prehe at 100U/mL tyrosinase solution. Add tyrosinase solution to tubes A and C again. After 40 minutes of reaction, quickly measure the absorbance value at 475 nm. The absorbance of tubes A, B, C, and D are recorded as A1, A2, A3, and A4, respectively. PBS solution served as the negative control, and Kojic acid solution (prepared with a pH 6.8 PBS solution) at 0.008 to 1.000 mg/mL was used as the positive control. The tyrosinase inhibition rate of the test samples was calculated using formula 1-1.

Table 1. Reagent ratio table of the experimental system

Reagent	A（μL）	B（μL）	C（μL）	D（μL）
L-tyrosine/L-dopa	800	800	800	800
The sample	0	0	800	800
PBS solution	1500	2500	700	1700
Tyrosinase	1000	0	1000	0

$$\text{Inhibition rate} = \frac{(A-B)-(C-D)}{A-B} \times 100\% \quad \text{1-1}$$

2.2.2 DPPH Free Radical Scavenging Activity

The antioxidant properties of niacinamide and phytocompound solution were evaluated using the DPPH test method. Weigh 20 mg DPPH, dissolve in anhydrous ethanol, and adjust to the mark in a 250mL volumetric flask to prepare a DPPH stock solution of 2×10^{-4} mol/L. In test tubes, mix equal volumes of the sample and anhydrous ethanol with the DPPH test solution to obtain tubes A_1 and A_2. Mix equal volumes of anhydrous ethanol with the sample to obtain tube A_3. The reaction is then carried out in the dark for 30 min, and the absorbance values of tubes A_1, A_2, and A_3 are measured at a wavelength of 517 nm. Anhydrous ethanol serves as the negative control, and 0.5 mg/mL vitamin C as the positive control. Three parallel samples were prepared for each test. The percentage of DPPH free radicals scavenged is calculated using formula 1-2.

$$\text{Inhibition rate（%）} = [（A_2+A_3）-A_1]/A_2 \quad \text{1-2}$$

2.2.3 Inhibition of Melanin Production in Melanoma Cells of B16-F10

The whitening effects of niacinamide and phytocompound solution were evaluated by measuring the changes in melanin content in B16-F10 mouse skin melanoma cells[11]. First, the density of the B16 cell suspension was adjusted to 5×10^4 cells/mL and then seeded into a 6-well plate at 2 mL per well, incubated at 37℃,5% CO_2 for 24 h. Meanwhile, samples were added to complete medium, filtered through a 0.22 μm membrane to obtain the test samples. The cell suspension in the well plate was removed and replaced with the test samples at a concentration of 2 mL per well, forming the sample group. The control group was prepared by adding complete medium and incubated at 37℃,5% CO_2 for an additional 48 h. After cultivation, the supernatant was discarded, and the cells were washed once with PBS, digested with trypsin, and collected by centrifugation. The cellsuspension was resuspended in 400 μL of 1M NaOH (10% DMSO), and incubated at 80℃ in a water bath for 3.5 h. The supernatant was then transferred to a 96-well plate and the absorbance values of each well

were measured at 405 nm. The experiment used 0.05% Kojic acid as the positive control, and the melanininhibition rate was calculated using formula 1-3.

$$M = (M_C - M_X) / M_C \times 100\% \quad 1\text{-}3$$

Where M -The inhibition rate of melanin production in B16 cells;Mx -The OD value of the sample group after the B16 cell experiment;Mc-The OD value of the negative control group after the B16 cell experiment[11].

2.2.4 LPS-induced macrophage model's inflammatory factor inhibitory activity

Cells in the logarithmic growth phase were seeded at 1 × 10^5 cells per well in a 96-well plate, with 200 μl per well. The plates were incubated overnight at 37℃ and 5% CO_2 in an incubator. Afterward, the culture medium was removed, and control, model, and sample + LPS groups were set up, with six replicates for each group. Except for the control group, samples containing 5 μg/mL LPS were added to each group, while the model group received 5% LPS culture medium and the control group received only the culture medium, with 200 μl per well. After 24 h of incubation, the supernatant was collected, sealed, and stored at refrigerated temperature. The levels of inflammatory factors TNF-α and 1L-1β were measured using a kit.

2.3Human Test Methods

The composition of niacinamide and phytocompound solution was formulated into an essence solution, and the whitening and spot-removing capabilities of the essence solution were tested using a UV-induced human skin darkening model. Thirty participants, aged between 21 and 48 years, were selected to meet the testing requirements. All human experiments were conducted under conditions of 21±1℃ temperature and 50±10% relative humidity.

2.3.1 UV-induced human skin black model test

Screen subjects with an ITA ° between 20 and 41, first determining the MED at the test site. Select each test area on the test site and use a sunlight simulator to irradiate the same point at 0.75 times the MED dose once daily for 4 consecutive days. The 4 days following the irradiation are the skin darkening period, during which no treatment is applied. On the 5th day after irradiation, visually assess the skin color of each test area using a skin colorimeter, and exclude any test areas with poor consistency (those with an ITA value differing by more than 5 from the homogenized values of all test areas). On the same day, apply the corresponding test substance to each darkened test area according to a random schedule. Continue applying for at least 4 weeks, and visually assess and instrumentally measure the skin color at 1 、 2 、 3 and 4 weeks after application, recording the ITA °and MI values separately. The darkened skin areas serve as negative controls, while products containing 7% ascorbic acid serve as positive controls. Each test area undergoes 3 parallel tests.

2.3.2 Facial skin chroma variation experiment

This study recruited 30 Chinese female subjects aged 30 to 50 with dull complexion, pigmentation, or yellowish skin. Subjects meeting any of the following criteria were excluded from participation: pregnant or lactating women, individuals with severe skin sensitivity, those requiring prolonged sun exposure due to occupational demands, participants who had undergone cosmetic treatments or facial surgeries within the past three months, and patients suffering from major illnesses or chronic conditions requiring long-term medication. During the testing period, subjects were instructed to use the test sample as a serum substitute after cleansing their face morning and evening for four consecutive weeks. Subjects were required to apply adequate sunscreen during the testing period and prohibited from using other whitening products.

Skin image acquisition and data measurement were conducted at fixed time points of 0, 1, 2, and 4 weeks. Prior to each test, subjects uniformly cleansed their faces and rested in the testing area for 30 minutes. The VISIA system (SB-003VISIA-CR) was used to capture initial skin images. Ten facial data collection points were selected (center of forehead, left eyebrow tail, right eyebrow tail, left eye corner, right eye corner, left cheekbone, right cheekbone, left mouth corner, right mouth corner, and chin). Skin da

ta (L, a, and b values) were measured using a skin testing instrument (Dermalab Seri es SkinLab Combo), while skin smoothness was measured using the VisioScan VC20 Plus. Three parallel measurements were set for each test point.

2.4 Data statistics

The test results were expressed by $\overline{X} \pm SD$. Paired *t*-test was used to compare the human body before and after, and independent sample *t*-test was used to compare the test area and control area. The regression coefficient (k value) of each parameter with the change of vacation was calculated at the same time. $p<0.05$ was considered to have statistical difference.

3. Results

The tyrosinase inhibition rate, DPPH free radical scavenging rate and hyaluronidase inhibition rate of sample 2 were the highest. After sample 2 was applied to B16F10 cells, the tyrosinase inhibition rate reached 78.93%, and the relative content of intracellular melanin was 25.20%. In the UV-induced human skin blackening model, the melanin content of the skin decreased by 12.29% and 21.46% after 2 and 4 weeks of the sample treatment, respectively. The results of 30 people's faces showed that after 14 days of use, the yellowness decreased by 18.7%, the redness decreased by 5.63%, the fineness increased by 10.21%, and the brightness increased by 17.59%.

3.1In vitro evaluation methods

3.1.1Tyrosinase inhibition rate

In vitro experimental results demonstrate that the nicotinamide and plant complex solution exhibits significant concentration-dependent inhibitory effects on tyrosinase activity. Among them, Sample 2 displayed the optimal inhibitory activity. Figure 1 illustrates the inhibitory activity of this complex on tyrosinase at various concentrations.

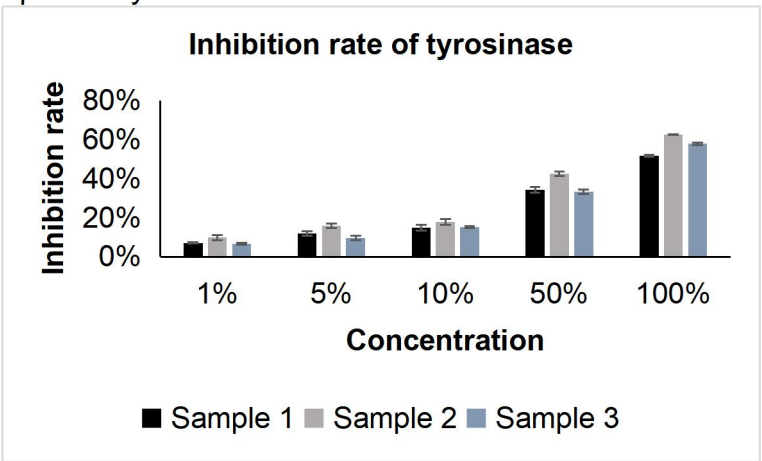


Figure 1. Tyrosinase inhibition rate of niacinamide + phytocompound solution.

3.1.2 DPPH free radical scavenging test

The research results indicate that all three solutions containing nicotinamide and plant complexes exhibited significant DPPH radical scavenging capacity, with Sample 2 dem onstrating the highest scavenging activity. The DPPH radical scavenging abilities of nic otinamide-plant complex solutions at different concentrations are shown in Figure 2.

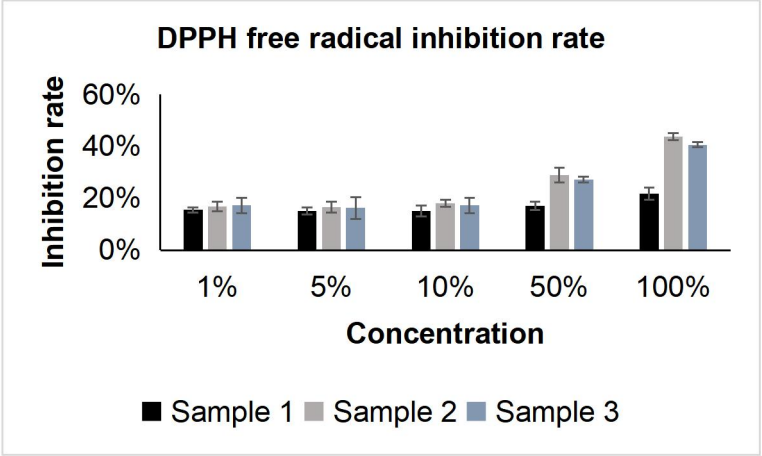


Figure 2. DPPH free radical scavenging rate of niacinamide and phytocompound solution

3.1.3 Inhibition of Melanin Production in Melanoma Cells of B16-F10

The experimental results show that 0.01% concentration of the nicotinamide and plant complex solution can significantly inhibit melanin production in B16-F10 cells, reducing melanin content to 49.60%. Similarly, 0.01% concentration of this solution has an inhib ition rate of about 57.97% on intracellular tyrosinase activity. The cytotoxicity, melanin content, and tyrosinase inhibition rates of different concentrations of nicotinamide and plant complexsolutions are shown in Figure 3 and 4.

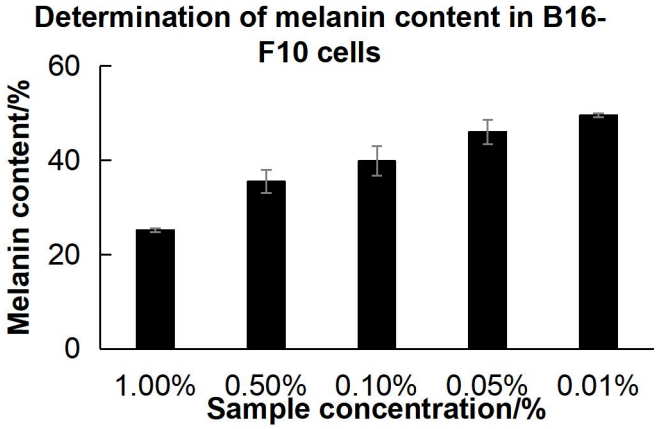


Figure 3. The melanin content in B16-F10 cells.

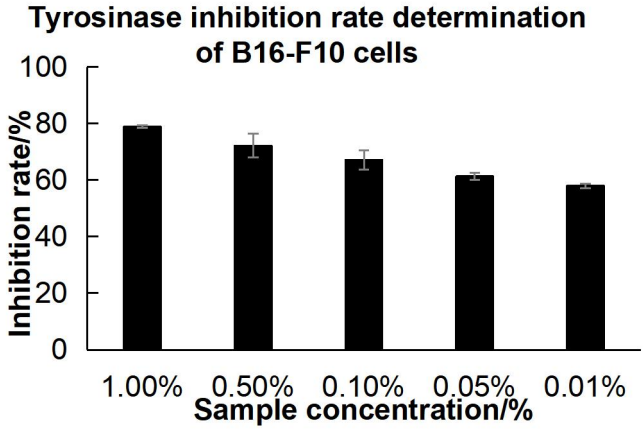
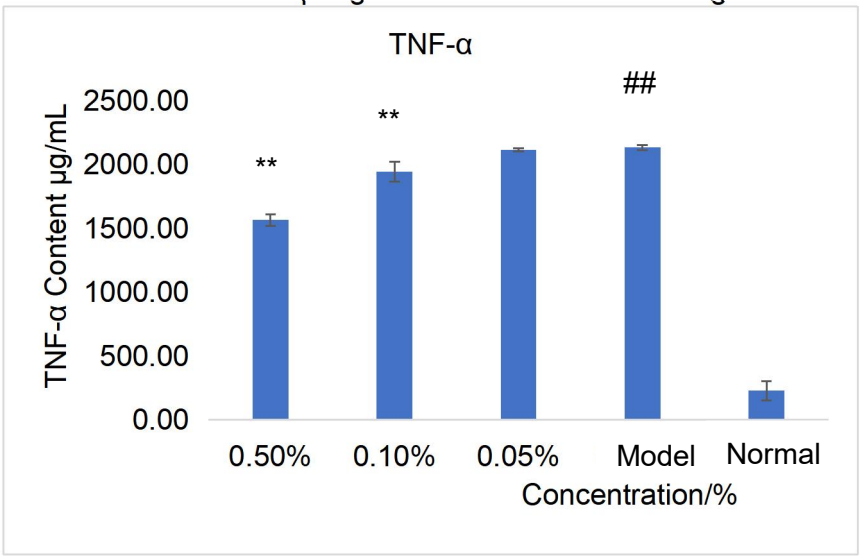


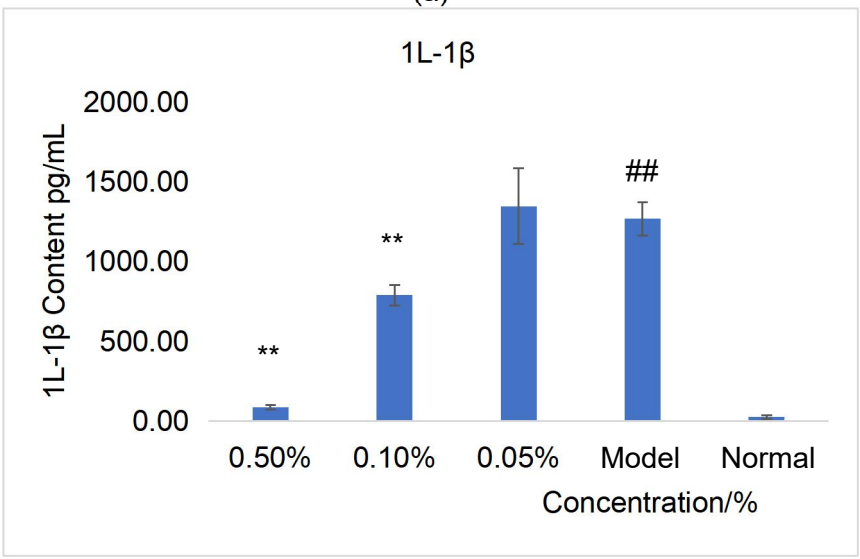
Figure 4. Tyrosinase inhibition rate determination of B16-F10 cells.

3.1.4 LPS-induced macrophage model's inflammatory factor inhibitory activity

The results of the cell model showed that the 0.1% concentration of essence solution had a highly significant inhibitory effect on the release of TNF-α and 1L-1β inflammatory factors, reducing them by 8.81% and 37.77%, respectively. The results of the inhibitory effects of different concentrations of essence solution on the release of inflammatory factors in the LPS-induced macrophage model are shown in Figure 5.



(a)



(b)

Figure 5. The effect of essence solution on inflammatory factors

(Figure (a) shows the changes in TNF-α content in the macrophage model; Figure (b) shows the changes in IL-1β content in the macrophage model)Where the "**" and "##" indicates $0.001<p<0.01$,The data is statistically significant）.

3.2 Human Test Methods

3.2.1 UV-induced human skin black model test

Compared to 7% ascorbic acid, the skin brightness has increased by by 17.58% and 31.56% after 2 weeks and 4 weeks of use, respectively, performing better than 7% ascorbic acid (13.18% and 22.32%, respectively); meanwhile, the melanin content in the skin decreased by 12.29% and 21.46%, also performing better than 7% ascorbic acid (9.43% and 15.90%, respectively). The changes in ITA° and MI values are shown in Table 2.

Table 2. Skin ITA°and MI results before and after using the essence

Testing	Test time	W0	W1	W2	W3	W4
ITA°	TP	26.84±5.08	29.71±4.14***	31.56±3.80***	33.56±4.00***	35.31±3.01***
	NC	27.82±4.71	28.03±4.26	28.41±3.79	28.67±3.76	28.84±3.76
	PC	26.93±4.48	28.63±4.36***	30.48±3.81***	32.02±4.25***	32.94±3.75***
MI	TP	244.93±29.70	228.87±27.70***	214.84±22.66***	204.97±21.20***	192.37±20.46***
	NC	236.31±28.83	236.63±27.61	236.84±25.49	236.07±25.93	236.07±26.30
	PC	245.80±27.93	233.71±28.37***	222.63±27.08***	215.21±24.90***	206.72±28.51***

Ps：All test results were expressed as $\overline{X} \pm SD$, compared with the negative control, "*" indicates 0.01<*p*<0.05, "**" indicates 0.001<*p*<0.01, and "***" indicates *p*<0.001.
Table legend:TP-Test product，NC-Negative control，PC-Positive control

3.2.2 Facial skin chroma changes

After two weeks of continuous use, the volunteers 'skin brightness showed a significant improvement, with the L* value increasing by 17.59%, the b* value across the face decreased by 18.67%, the a* value across the face decreased by 5.63%, the volunteers' skin roughness decreased by 10.21%. The changes in skin values for the 30 volunteers over two weeks are shown in Figure 6.

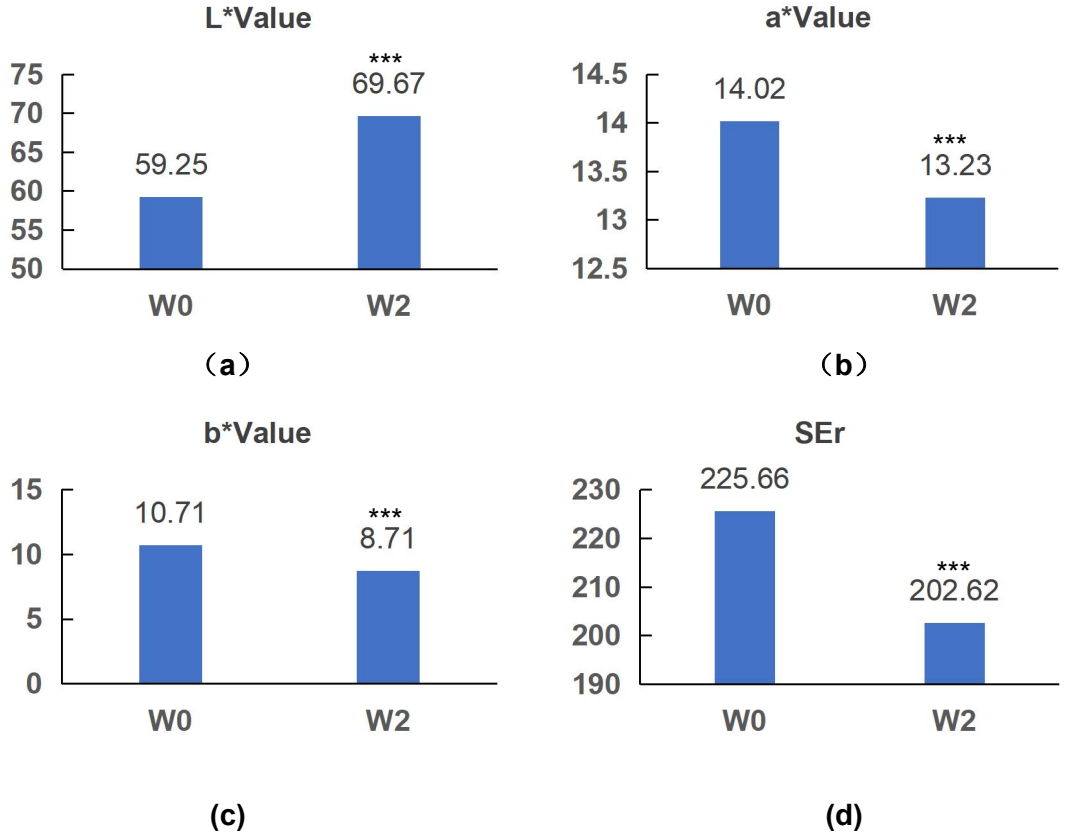


Figure 6. Changes in L, a, b, and SEr values of facial skin after using the essence

((a)The change in skin L* value represents skin lightness;(b)The change in skin a* value represents skin erythema; (c)The change in skin b* value represents skin yellowness Chromacity; (d)The change in skin SEr represents Smoothness parameter)
"*" indicates 0.01<*p*<0.05, "**" indicates 0.001<*p*<0.01, and "***" indicates *p*<0.001.

3.2.3 Case pictures of skin color changes in some volunteers

Following two weeks of continuous use, the volunteers exhibited a significant reduction in facial pigmentation spots. Additionally, improvements in skin erythema were observed. The overall facial skin tone became more uniform and delicate, with a concurrent significant increase in skin tone brightness. The comparison effect of the volunteers' faces before and after is shown in Figure 7.

Figure 7. Changes in facial features of volunteers before and after VISIA photography

4. Discussion

In recent years, consumer demands for brightening products have evolved to become more specific and sophisticated, with increasing emphasis on rational whitening approaches, naturally luminous results, and gentle yet effective formulations.

Nicotinamide is a well-established brightening agent that effectively inhibits melmanin synthesis and blocks its transfer from melanocytes to keratinocytes.

This study delves into the multifaceted causes of skin pigmentation, combining nicotinamide with traditional Chinese botanical extracts in a synergistic formulation. The resulting multi-target system delivers comprehensive benefits, including:skin brightening and hydration, anti-inflammatory, anti-glycation, anti-antioxidant protection and strengthened skin barrier function. Among these traditional Chinese plant active ingredients,glycyrrhiza uralensis (licorice) root extract contains components such as glycyrrhizin and glycyrrhizin flavonoids, which can inhibit tyrosinase activity, suppress melanin production, and have a synergistic anti-inflammatory effect at the same time[12,13].The main components of artemisia capillaris extract are flavonoids and coumaric acid, which have a good effect in eliminating free radicals and inhibiting tyrosinase. Scutellaria baicalensis root extract contains components such as baicalin and baicalin, which can resist ultraviolet rays and inhibit inflammatory factors[14,15].The lycium barbarum polysaccharides in lycium chinense fruit extract can enhance the activity of SOD in the skin, inhibit the oxidative pathway of melanin production, and at the same time provide the skin with amino acids, vitamins and other nutrients, promote the vitality of keratinocytes, creating a good complexion[16].Finally, the bark of morus alba bark extract contains components such as morin, which can eliminate free radicals, have antioxidant effects, and synergistically inhibit inflammatory factors.The above-mentioned traditional plant active ingredients can comprehensively enhance the whitening effect of nicotinamide, creating a healthy and radiant complexion. It has a promising application prospect in the fields of skin whitening, anti-aging and natural skin care.

5. Conclusion

This study initially determined the optimal ratio of nicotinamide to plant complexes via biochemical assays. Subsequently, the whitening and anti-inflammatory efficacy of this composition solution was validated through in vitro cellular experiments. Finally, it is prepared into an essence liquid and human tests are conducted.In the B16 cell model, the tyrosinase inhibition rate of the composition solution reached 78.93%, and the relative content of intracellular melanin was 25.20%. In the macrophage model, the 0.5% and 0.1% composition solution significantly inhibited the release of the inflammatory factors TNF-α and IL-1β. Specifically, TNF-α release was reduced by 26.67% and 8.81%, while IL-1β release was decreased by 93.12% and 37.77%, respectively. In the UV-induced human skin blackening model, the melanin content of the skin decreased by 12.29% and 21.46% after 2 and 4 weeks of the sample treatment, respectively. The results of 30 people's faces showed that after 14 days of use, the yellowness decreased by 18.7%, the redness decreased by 5.63%, the fineness increased by 10.21%, and the brightness increased by 17.59%. The essence liquid compounded niacinamide and phytocompound can synergistically improve skin tone.

References:

[1] Yakimov B P, Shirshin E A, Schleusener J. et al. Melanin distribution from the dermal–epidermal junction to the stratum corneum: non-invasive in vivo assessment by fluorescence and Raman microspectroscopy[J]. Sci Rep , 2020, 10, 14374.

[2] UYEN L D P, NGUYEN D H, KIM E-K. Mechanism of skin pigmentation [J]. Biotechnology and Bioprocess Engineering, 2008, 13(4): 383-95.

[3] HAKOZAKI T, MINWALLA L, ZHUANG J, et al. The effect of niacinamide on reducing cutaneous pigmentation and suppression of melanosome transfer [J]. British Journal of Dermatology, 2002, 147(1): 20-31.

[4] BISSETT D L, OBLONG J E, BERGE C A. Niacinamide: A B Vitamin that Improves Aging Facial Skin Appearance [J]. Dermatologic Surgery, 2005, 31(s1): 860-6.

[5] WAHAB S, ANNADURAI S, ABULLAIS S S, et al. Glycyrrhiza glabra (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology [J/OL] 2021, 10(12):

[6] WANG Y-S, CHO J-G, HWANG E-S, et al. Enhancement of Protective Effects of Radix Scutellariae on UVB-induced Photo Damage in Human HaCaT Keratinocytes [J]. Applied Biochemistry and Biotechnology, 2018, 184(4): 1073-93.

[7] RAMAN ST G A, CHEN C, JIN C, LI SH, CHEN HJ, ET AL. In vitro and In vivo antioxidant activity of flavonoid extracted from mulberry fruit (Morus alba L.) [J]. Phcog Mag, 2016, 12(128-33.

[8] DENG H-B, CUI D-P, JIANG J-M, et al. Inhibiting effects of Achyranthes bidentata polysaccharide and Lycium barbarum polysaccharide on nonenzyme glycation in D-galactose induced mouse aging model [J]. Biomed Environ Sci, 2003, 16(3): 267-75.

[9] POLCARO L M, CERULLI A, MONTELLA F, et al. Chemical Profile and Antioxidant and Tyrosinase Inhibitory Activity of Chamaemelum nobile L. Green Extracts [J/OL] 2024, 11(3):

[10] He Yifan , Suyama Takashi L. , Kim Hyunwoo , et al. Discovery of Novel Tyrosinase Inhibitors From Marine Cyanobacteria[J].Frontiers in Microbiology, 2022, 13.
[11] Wu P Y, You Y J, Liu Y J, et al. Sesamol Inhibited Melanogenesis by Regulating Melanin-Related Signal Transduction in B16F10 Cells[J]. Int. J. Mol. Sci. 2018, 19, 1108.
[12] Wahab S, Annadurai S, Abullais S S, et al. Glycyrrhiza glabra (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology[J]. Plants, 2021, 10, 2751.
[13] Zheng, Y, Wang H, Yang M, et al. Prenylated Flavonoids from Roots of Glycyrrhiza uralensis Induce Differentiation of B16-F10 Melanoma Cells[J]. Int. J. Mol. Sci. 2018, 19, 2422.
[14] Liau P R, Wu M S, Lee C K. Inhibitory Effects of Scutellaria baicalensis Root Extract on Linoleic Acid Hydroperoxide-induced Lung Mitochondrial Lipid Peroxidation and Antioxidant Activities[J]. Molecules 2019, 24, 2143.
[15] Ali A, Kim E H, Lee J H, et al. Processed Scutellaria baicalensis Georgi Extract Alleviates LPS-Induced Inflammatory and Oxidative Stress through a Crosstalk between NF-κB and KEAP1/NRF2 Signaling in Macrophage Cells[J]. Appl. Sci. 2021, 11, 6055.
[16] Shang Y F , Hao W D , Ni Z J, et al. Lycium barbarum ethanol extraction: Application as cosmetic product ingredients[J]. Industrial Crops and Products, 2023, 202, 117130.