IFSCC2025-849

"Unlocking the Anti-Wrinkle and Repair Power Camellia Japonica Seed Extract: Insights from Cell Studies"

Fu Haokun [1], Chen Dandan [1], Lu Xuelian [1], Luo Yu [1], Yin Shipeng [1], Li Guangtao [1] and Gao Hongqi [1]

[1] Research and Innovation Center, Shanghai Forest Cabin Biological-tech Co., Ltd., Shanghai, China

1. Introduction

With the rise of aging societies and the rapid growth of the functional cosmetics market, the demand for anti-wrinkle and skin-repairing products is steadily increasing [9]. As a result, brands are actively developing targeted formulas to meet these needs. At the same time, a new wave of consumer interest has reshaped the beauty industry. "Green beauty" is now a dominant trend. Consumers are seeking safe, natural, and sustainable cosmetic products that reflect both personal well-being and environmental responsibility [3]. This growing preference has brought natural plant-based ingredients to the forefront of skincare research.

Skin aging is a visible and complex process, driven by intrinsic factors such as genetics and extrinsic factors like UV exposure and pollution. Over time, the epidermis becomes thinner, collagen and elastin fibers break down, and the skin's repair ability weakens. While this process is inevitable, it can be delayed or improved with proper skincare. Among various options, natural extracts have become highly popular for their multi-targeted effects and safety [11].

Plant ingredients have long been used for skincare across cultures [1]. They are rich in active compounds, such as polyphenols, fatty acids, and terpenoids, which help fight oxidative stress, reduce inflammation, and support the skin barrier [4, 10]. Among them, Camellia species are gaining attention for their broad skin benefits. There are research findings indicate that Camellia seeds, leaves, and flowers contain a wide range of bioactive compounds that could be beneficial in skin repair and aging prevention [2, 5, 7].

In East Asia, *Camellia Japonica* seed oil has long been valued in traditional skincare for its calming and moisturizing effects [8]. Recent research has further revealed that extracts from *Camellia japonica* seeds possess antioxidant and anti-inflammatory properties, showing promise to combat skin aging [6].

In this study, we introduced a novel *Camellia Japonica* seed extract (CJSE) and focused on its efficacy. We aimed to explore its potential in promoting skin repair and anti-wrinkle effects using in vitro models. By understanding its biological activities, we hope to offer insights into the development of natural, effective, and safe skincare formulations aligned with market trends and consumer values.

Chapter 2 introduces the materials and methods used in the study. The results are presented in Chapter 3 and discussions are in Chapter 4. Chapter 5 is the conclusion part.

2. Materials and Methods

2.1 Materials

CJSE, HSF cells, HaCaT cells, High-glucose DMEM culture medium, trypsin, phosphate-buffered saline (PBS), dual antibodies, fetal bovine serum (FBS), 4% paraformaldehyde, Tween 20, immunostaining permeabilization solution (Triton X-100), bovine serum albumin (BSA), anti-FLG primary antibody (rabbit anti-), fluorescent-labeled secondary antibody (rabbit anti-), 4',6-diamidino-2-phenylindole (DAPI), sodium dodecyl sulfate (SDS).

2.2 Methods

2.2.1 Preparation of CJSE

Camellia seed powder was extracted using 80% ethanol at a solid-to-liquid ratio of 1:20 (g/mL), followed by 30 minutes of ultrasonication and 3 hours of stirring at 60°C. The extract was filtered, and the filtrate was concentrated tenfold to remove ethanol. After reconstitution with water to the original volume, the solution was loaded onto a YKDH-5 resin column (1.5 bed volumes, flow rate: 1.0 BV/h). The column was rinsed with 2 BV of water and eluted with 3 BV of 60% ethanol. The eluate was concentrated to obtain Extract A.

The residual solid was dried at 60°C and extracted with water under the same solid-to-liquid ratio. Extraction included 30 minutes of ultrasonication, 2 hours at 60°C, and 1 hour at 90°C. After filtration, 0.5% (w/w) activated carbon was added for decolorization at 60°C for 30 minutes, followed by filtration to obtain Extract B.

Extracts A and B were combined to produce the final CJSE.

2.2.2 Type III Collagen Expression

Frozen HSF cells were thawed and seeded into culture flasks at an appropriate density. The cells were cultured in DMEM medium containing 10% FBS at 37 °C with 5% CO_2 in a cell incubator. HSF cells in the logarithmic growth phase were washed twice with PBS and then digested with trypsin. The digestion was stopped by adding DMEM medium containing 10% FBS. After centrifugation, the supernatant was discarded, and the cells were resuspended in DMEM medium (10% FBS) to prepare a single-cell suspension. Then, 2 mL of the cell suspension was added to each well of a 6-well plate containing coverslips, and the cells were cultured in an incubator.

When the cell confluency reached approximately 50%, the model group was treated with 0.25 μM doxorubicin for 48 hours. At the same time, the sample group (0.3% CJSE) was treated with the test concentration under the same conditions.

For the immunofluorescence slide preparation, each well was fixed with 1 mL of 4% paraformaldehyde at room temperature for 20 minutes. Cells were then permeabilized with 0.5% Triton X-100 for 15 minutes at room temperature. After washing with PBS, the samples were blocked at room temperature for 1 hour, and the blocking solution was then removed. The primary antibody was incubated overnight at 4 °C, followed by incubation with the fluorescent secondary antibody at 37 °C for 1 hour in the dark. After washing with PBS, the cells were incubated with DAPI for 5 minutes at room temperature in the dark. After treatment, coverslips were removed and fixed overnight using mounting medium. Fluorescence microscopy was used to capture images, and data were collected for analysis.

2.2.3 MMP-1 Expression

The procedures for cell culture and seeding were the same as described in section 2.2.2. UV irradiation was used to induce the model. Then, 2 mL of DMEM medium containing 0.3% sample was added to each well, and the cells were further incubated at 37 °C with 5% CO_2 for 48 hours. The immunofluorescence slide preparation was performed following the same procedure as described in section 2.2.2. Fluorescence images were captured under a fluorescence microscope, and data analysis was performed.

2.2.4 Cell Migration

HaCaT cells preserved in liquid nitrogen were seeded into culture flasks at an appropriate density. DMEM medium containing 10% FBS was added, and the cells were incubated at 37°C in a 5% CO_2 incubator. The procedures for cell seeding were the same as described in section

2.2.2. A volume of 2 mL of the suspension was added to each well of a 6-well plate. The cells were incubated in a cell incubator for 24 hours. 10 μL of pipehead was used to scratch. After washing twice with PBS, 2 mL of DMEM medium containing 0.3% sample was added to the well. A blank control group with DMEM medium only was also set up. After 24 hours, images of the same field were taken for the sample group. The scratch spacing (d) at two locations at each time point were measured and cell mobility was calculated, as follows:

$$\text{Cell mobility}\ (\%) = \frac{d_{0h} - d_{24h}}{d_{0h}} \times 100\%$$

2.2.5 Filaggrin Expression

The procedures for cell culture and seeding were the same as described in section 2.2.4. The culture medium was removed, and, except for the blank control group, 2 mL of DMEM medium (10% FBS) containing 30 μg/mL SDS and 0.3% sample concentration was added to each well. The cells were then incubated at 37°C with 5% CO_2 for 48 hours. The immunofluorescence slide preparation was performed following the same procedure as described in section 2.2.2. Fluorescence images were captured under a fluorescence microscope, and data analysis was performed.

2.2.6 Statistical Analysis

Statistical analysis was performed using WPS Office. Data are presented as mean±standard error of the mean (SEM). Normality of data distribution was assessed using histograms. For normally distributed data, T-test statistical analysis was conducted with a significance level of α = 0.05. For non-normally distributed data, the Wilcoxon test was applied, with α = 0.05 considered statistically significant. “*” represents “p <0.05”, “**” represents “p <0.01”, “***” represents “p <0.001”.

3. Results

3.1 Type III Collagen Expression

Type III collagen plays a key role in maintaining skin structure and elasticity. After 48 hours of treatment with 0.3% CJSE, the relative expression of type III collagen in HSF cells increased by 33.43% compared to the control group (Table 1, Figure 1 and 2), indicating a highly significant promotion of type III collagen expression. Under the experimental conditions, CJSE demonstrates anti-wrinkle potential.

Table 1 The relative expression of type III collagen in HSF cells following treatment with CJSE

	Control group	**0.3% CJSE**
Relative expression (%)	100.00 ± 0.41	133.43 ± 1.95***

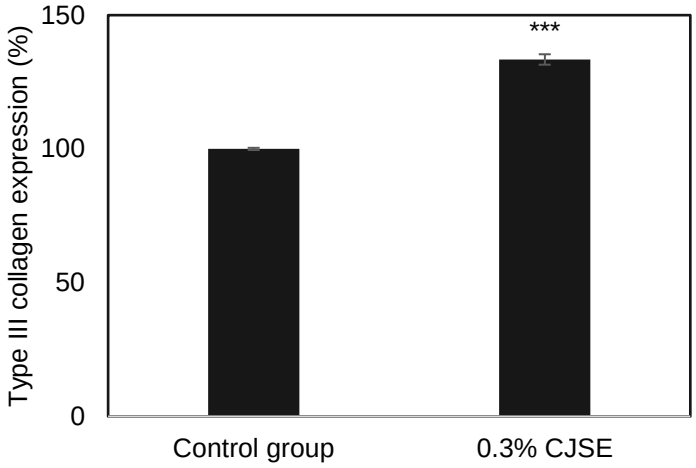

Figure 1 The relative expression of type III collagen in HSF cells following treatment with CJSE

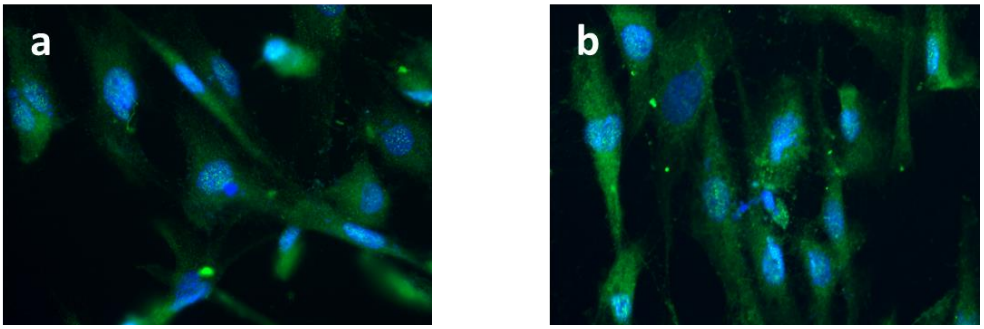

Figure 2 The fluorescence micrographs of type III collagen expression in HSF cells of (a) control group, (b) 0.3% CJSE at 48h

3.2 MMP-1 Expression

Matrix metalloproteinase-1 (MMP-1) is an enzyme that breaks down collagen in the skin. Its over-expression is closely linked to wrinkle formation. After 48 hours of treatment with 0.3% CJSE, the relative expression of MMP-1 in HSF cells decreased by 13.27% compared to the control group (Table 2, Figure 3 and 4). This indicates a highly significant inhibitory effect on MMP-1 expression. Under these experimental conditions, CJSE exhibits anti-wrinkle efficacy.

Table 2 The relative expression of MMP-1 in HSF cells following treatment with CJSE

	Control group	**0.3% CJSE**
Relative expression (%)	100.00 ± 0.74	86.73 ± 0.34***

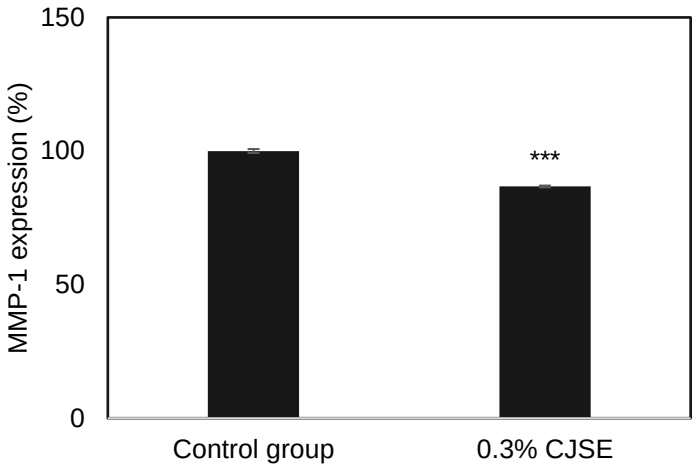

Figure 3 The relative expression of MMP-1 in HSF cells following treatment with CJSE

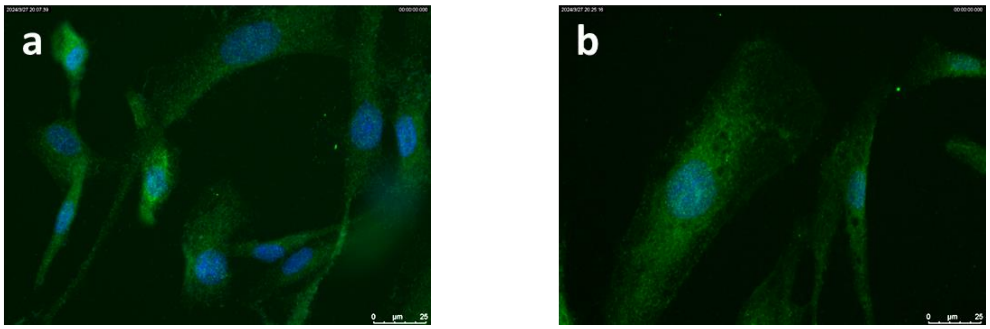

Figure 4 The fluorescence micrographs of MMP-1 expression in HSF cells of (a) control group, (b) 0.3% CJSE at 48h

3.3 Cell Migration

After 24 hours of treatment with 0.3% CJSE, the migration rate of HaCaT cells increased to 32.31±1.65%, compared to 25.64±0.40% in the blank control group, representing an increase of 6.67% (Table 3, Figure 5 and 6). This significant enhancement in cell migration indicates that CJSE promotes HaCaT cell migration under the experimental conditions, demonstrating its potential skin-repairing efficacy.

Table 3 Impact of 24-hour CJSE exposure on HaCaT cell migration

	Control group	**0.3% CJSE**
Cell mobility (%)	25.64 ± 0.40	32.31 ± 1.65**

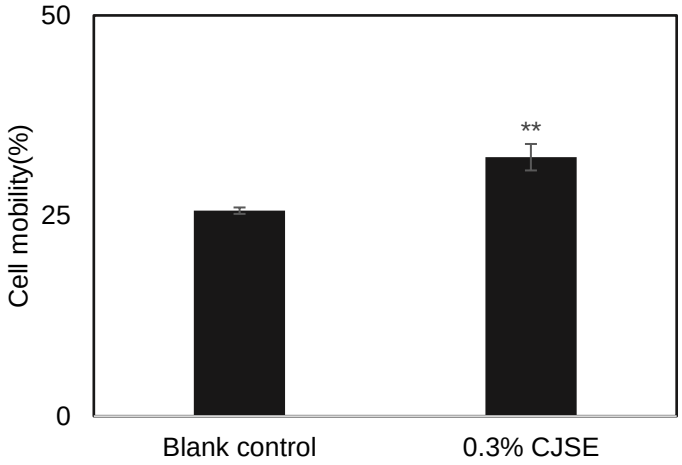

Figure 5 Impact of 24-hour CJSE exposure on HaCaT cell migration

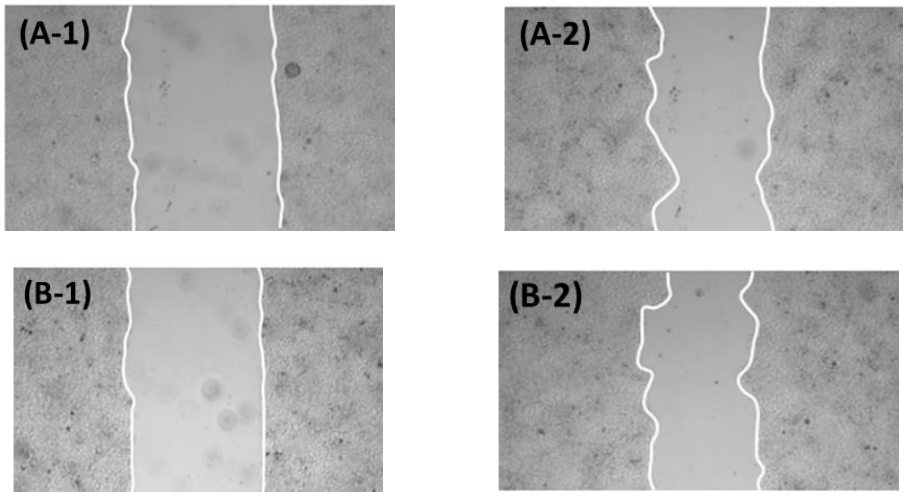

Figure 6 The fluorescence micrographs of cell migration of (A) blank control, (B) 0.3% CJSE at (1) t0h and (2) t24h

3.4 Filaggrin Expression

After treatment with 0.3% CJSE, the relative expression of filaggrin in HaCaT cells increased to 143.29±5.46%, compared to 100.00±1.01% in the control group, representing a 43.29% increase (Table 4, Figure 7 and 8). This significant upregulation indicates that 0.3% CJSE effectively promotes filaggrin expression in HaCaT cells, suggesting its skin-repairing potential under the experimental conditions.

Table 4 The relative expression of filaggrin in HaCaT cells following treatment with CJSE

	Control group	**0.3% CJSE**
Relative expression (%)	100.00 ± 1.01	143.29 ± 0.21***

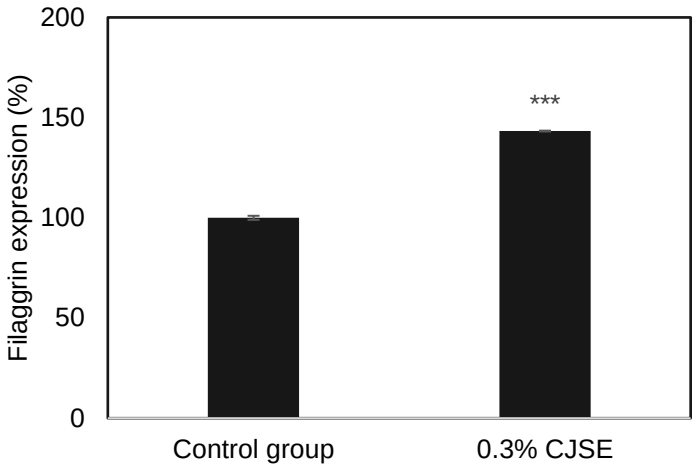

Figure 7 The relative expression of filaggrin in HaCaT cells following treatment with CJSE

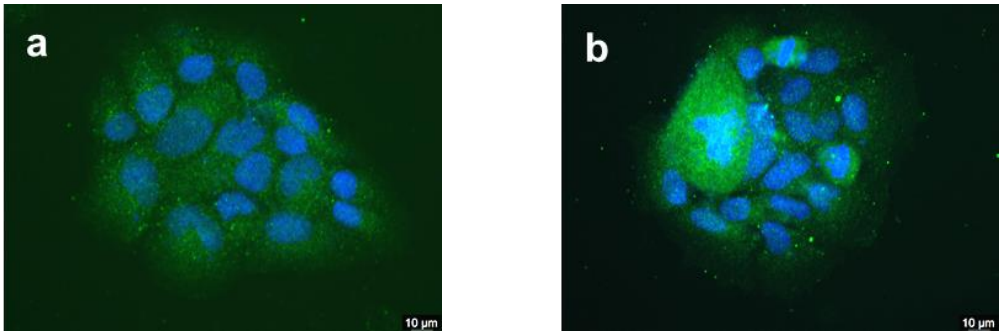

Figure 8 The fluorescence micrographs of filaggrin expression in HaCaT cells of (a) control group, (b) 0.3% CJSE

4. Discussion

In previous studies, Camellia leaf extract has been proved to improve skin firmness and reduce wrinkle formation [6]. This study demonstrates that 0.3% CJSE has both anti-wrinkle and skin-repairing effects. The observed 33.43% increase in type III collagen expression in HSF cells suggests that the extract significantly enhances collagen synthesis. This aligns with earlier findings that collagen production is crucial for maintaining skin structure and elasticity, especially in aging skin [1].

The extract also reduced MMP-1 expression by 13.27%, which is notable since MMP-1 is known to degrade collagen and accelerate skin aging. Previous research has shown that plant-derived antioxidants can suppress MMP expression [4, 7], and the results are consistent with those studies. This adds further evidence that the extract protects skin structure not only by promoting collagen production but also by inhibiting its breakdown, reinforcing its anti-aging potential.

In terms of skin repair, cell migration plays a key role in wound healing. The extract improved HaCaT cell migration by 6.67%, suggesting that it may help accelerate skin regeneration. Filaggrin expression in HaCaT cells increased by 43.29% after treatment. As a major protein in the skin barrier, filaggrin contributes to skin hydration and defense.

Together, these results suggest that CJSE works through multiple mechanisms to maintain youthful skin. It promotes collagen synthesis, inhibits collagen degradation, supports cell migration, and boosts barrier-related protein expression. These dual effects make it a promising active ingredient for cosmetic products targeting both skin aging and repair.

Future studies could further investigate its efficacy in more complex skin models or clinical settings. Exploring the mechanisms behind its regulatory effects on gene and protein expression may also provide valuable insights for optimizing its use in skincare formulations.

5. Conclusion

In this study, the novel CJSE demonstrated strong anti-wrinkle and skin-repairing properties. It significantly enhanced the expression of type III collagen in human dermal fibroblasts (HSF), a key structural protein responsible for maintaining skin elasticity and firmness. At the same time, it effectively suppressed the expression of MMP-1, an enzyme involved in collagen degradation. In keratinocyte (HaCaT) cells, the *Camellia Japonica* extract promoted cell migration, an essential process for skin wound healing, and significantly upregulated filaggrin expression, a critical component of the skin barrier that helps retain moisture and protect against external stressors. These results collectively suggest that CJSE holds great potential as a multifunctional cosmetic ingredient. By supporting collagen synthesis, preventing collagen breakdown, enhancing skin repair, and strengthening the barrier function, it presents a promising solution for developing advanced formulations targeting signs of aging and promoting overall skin health.

Acknowledgments

The authors thank Shanghai Forest Cabin Biological-tech Co., Ltd. for funding this project.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

References

[1]Binic, I., Lazarevic, V., Ljubenovic, M., Mojsa, J., & Sokolovic, D. (2013). Skin ageing: natural weapons and strategies. Evidence-Based Complementary and Alternative Medicine, 2013(1), 827248.

[2]Choi, M. H., Min, M. J., Oh, D. S., & Shin, H. J. (2013). Antimicrobial and antioxidant activity of Camellia japonica extracts for cosmetic applications. KSBB Journal, 28(2), 99-105.

[3]Faria-Silva, C., Ascenso, A., Costa, A. M., Marto, J., Carvalheiro, M., Ribeiro, H. M., & Simões, S. (2020). Feeding the skin: A new trend in food and cosmetics convergence. Trends in Food Science & Technology, 95, 21-32.

[4]Ferreira, M. S., Magalhães, M. C., Oliveira, R., Sousa-Lobo, J. M., & Almeida, I. F. (2021). Trends in the use of botanicals in anti-aging cosmetics. Molecules, 26(12), 3584.

[5]Guo, J., Luo, J., Zhou, Y., Liu, H., & Jin, D. (2024). Active components and skin care properties of tea seed oil from Camellia sinensi. BioResources, 19(4), 716.

[6]Kim, H. T., Kim, T. J., Kim, E. H., & Kwak, B. M. (2020). Anti-oxidant and anti-aging effect of extracts from seed of Camellia japonica L. by supercritical fluid extraction. The Journal of Korean Medicine Ophthalmology and Otolaryngology and Dermatology, 33(4), 32-43.

[7]Kim, J. E., Ko, Y. R., Boo, S. H., Kang, S. H., & Lee, N. H. (2022). Anti-inflammatory and anti-oxidative activities for the subcritical water extract of Camellia japonica flowers. Journal of the Society of Cosmetic Scientists of Korea, 48(2), 97-104.

[8]Kim, S. B., Jung, E. S., Shin, S. W., Kim, M. H., Kim, Y. S., Lee, J. S., & Park, D. H. (2012). Anti-inflammatory activity of Camellia japonica oil. BMB reports, 45(3), 177-182.

[9]Lee, J. Y., & Lee, J. M. (2021). Analysis of Rcent Research Trends in Development of Functional Cosmetic Materials for Wrinkle Improvement. Journal of Convergence for Information Technology, 11(6), 181-187.

[10]Pereira, A. G., Garcia-Perez, P., Cassani, L., Chamorro, F., Cao, H., Barba, F. J., ... & Prieto, M. A. (2022). Camellia japonica: A phytochemical perspective and current applications facing its industrial exploitation. Food chemistry: X, 13, 100258.

[11]Xie, M., Jiang, Z., Lin, X., & Wei, X. (2024). Application of plant extracts cosmetics in the field of anti-aging. Journal of Dermatologic Science and Cosmetic Technology, 100014.