## **Preparation and Stability of Moisturizing Essence Liquid from Trollius Chinensis Bunge Stem Extract**

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Corresponding Author: Yang Liu School of Biology and Food Engineering, Changshu Institute of Technology, Changshu, China Email: liuyang84@126.com Abstract: The stem, a byproduct of traditional Chinese herbal medicine Trollius chinensis Bunge, has not been effectively developed and utilized for a long time. In this study, the polysaccharides and flavonoids from Trollius chinensis Bunge was used as main ingredient in moisturizing essence liquid, and the preparation and stability of moisturizing essence liquid was studied. The approach was as follows: The main active ingredients, including the compound extract of polysaccharides and flavonoids from the stem of Trollius chinensis Bunge, 1, 2-propanediol, sodium hyaluronate, glycerol and rose hydrosol were used as single factors, the orthogonal test method was used to optimize the formula by moisturizing retention rate. The preparation and stability of moisturizing essence liquid were also determined. The results showed that the optimum formula of essence liquid was as follows: Polysaccharides and flavonoids compound extract of TC stem 2.5%, 1,2propanediol 6%, glycerol 4%, sodium hyaluronate 0.6%, rose hydrosol 4%. The moisturizing retention rate was up to 99.30%, which was significantly higher than the control products. The essence liquid demonstrated improved cold and heat resistance, showing no delamination after centrifugation. The optimized essence liquid exhibited an advantage in the moisture retention rate test compared with the control products. This study lays the application foundation for the development and utilization of the waste Trollius chinensis Bunge stem.

Keywords: Trollius Chinensis Bunge, Polysaccharides, Flavonoids, Essence Liquid, Moisture

### Introduction

Trollius Chinensis Bunge (TC), a member of the Ranunculaceae family, is rich in active components, comprising flavonoids, polysaccharides, primarily alkaloids and organic acids (Liu et al., 2016; Wang et al., 2020). According to existing research on the main constituents of TC, flavonoids and polysaccharides are critical active ingredients. Flavonoids are polyphenolic compounds involved in plant secondary metabolism, exhibiting multiple biological activities (Hollman and Katan, 1999; Habtemariam et al., 1997). Relevant pharmacological and clinical trials have confirmed that flavonoids have a wide range of pharmacological functions (Habtemariam et al., 1997; Hu et al., 1994). TC polysaccharide is a type of miscellaneous polysaccharide that belongs to the category of plant polysaccharides. TC exhibits various biological activities such as moisturizing, anti-tumor, anti-aging, immune regulation and blood sugar lowering. It also has the advantages of low toxicity and less residue. Recent research has confirmed that plant polysaccharides possess good film-forming and waterabsorbing properties and can be well applied in moisturizing cosmetics (Sebti and Coma, 2002; Li, 2017). Polysaccharides can also enhance the activity of some antioxidantantioxidant enzymes, such as SOD, GSH Px, etc., thereby playing an anti-aging role (Lee et al., 2003). For example, Lycium barbarum polysaccharides can inhibit the activity of tyrosinase and reduce skin melanin production (Liu et al., 2018)., while fucoidan helps cells produce various growth factors (Robert et al., 2003). It was found that based on 10 mg/mL silk fibroin oligopeptide, different concentrations of Cordyceps militaris polysaccharides were combined, and the moisturizing, anti-UV and antioxidantantioxidant abilities of the complex were significantly enhanced after skin care characteristic testing (Zhao, 2019). In addition, numerous studies have shown that plant flavonoids and polysaccharides play a significant role



in various aspects such as antioxidation (Ma *et al.*, 2020), anti-tumor (Luo *et al.*, 2022; Oliveira *et al.*, 2019), anti-fatigue (Liu *et al.*, 2021), anti-aging (Shan *et al.*, 2022; Fournière *et al.*, 2021), antibacterial, antiviral (Patel and Kumari, 2022; Agregán *et al.*, 2023), hypoglycemic and lipid-lowering effects (Wang *et al.*, 2022; Yu *et al.*, 2020).

Currently, research on TC domestically focused on exploring the active ingredients in the petals, while research on the stems was relatively lacking, leading to low utilization of experimental raw materials and high experimental costs. Previous research found that the stems of TC have chemical components similar to those of petals (Liu et al., 2017; Wang et al., 2013) and the polysaccharides and flavonoids in TC stem were compounded in a certain proportion, exhibiting superior moisturizing and antioxidant activity (Zhang et al., 2022). This research outcome provides valuable insights into the potential application of TC stem extract in cosmetic product development. The study suggests that TC stem extract could serve as an effective natural compound for moisturization, which aligns with the current trend in cosmetic formulations favoring natural plant extracts over synthetic alternatives. Indeed, the application of TC stem extract in the cosmetics industry could represent a significant opportunity for both resource optimization and economic benefit. Currently, the dried petals of TC are highly valued and widely used in pharmaceutical production, fetching prices as high as 30-50 USD per kilogram. In contrast, the stems are often discarded as waste, representing an underutilized resource. If the TC extract can be applied in the cosmetics industry, it will be beneficial for the in-depth development and utilization of TC stem resources. It can increase farmers' income and provide a new low-cost source of raw materials for the cosmetics industry. However, the moisturizing effect of the TC stem extract applied in cosmetics must be addressed before its widespread adoption.

Therefore, this study mainly discussed how to develop an essence liquid with a good moisturizing effect using the polysaccharides and flavonoids compound of TC stem as the main active materials. This study may provide an application foundation for the development and utilization of waste stemming from TC and enhance its added value.

## **Materials and Methods**

# *Extraction Process of Polysaccharides and Flavonoids from TC*

In this experiment, the water extraction method was employed to extract polysaccharides from the stems of TC (TCS, obtained from the market in Chengde City of Hebei Province, China, in 2023). The detailed protocol was based on the experimental method described by Qian *et al.* (2022); Li *et al.* (2015). The preparation process of essence liquid is shown in Fig. (1). The TCS were ground through a 40 mesh sieve as raw material. The extraction methods for polysaccharides and flavonoids have been explored in previous studies, so the optimal extraction process was directly selected based on the findings of the earlier research (Zhang *et al.*, 2022).

The specific operation of polysaccharides was as follows: The solid-liquid ratio is 1:60 (g/mL), and the extraction was conducted using a water bath (supplied by Shanghai Boxun Industrial Co., Ltd., HH. S11-1) at 80°C for 3 (h), with 3 times. The extraction solution was filtered and concentrated by a rotary evaporator (RE-Shanghai Zhengqiao Factory Scientific 5220A, Instrument Co., Ltd.) to 1/5 of the original volume. Ethanol solution (95%, v/v) was added to the concentrated solution of TC stem until the alcohol concentration reached 80% (v/v) and stored under 4°C for 24-48 (h). After alcohol precipitation, they were centrifuged for 10 min by a centrifuge (LDC-5, 4000 xg, Beijing Lei Zhenxing Instrument Co., Ltd). After centrifugation, the sediment was dried by a vacuum freeze dryer (LGJ-12 Beijing Songyuan Huaxing Technology Development Co., Ltd) to obtain the crude polysaccharide powder from the TC stem.

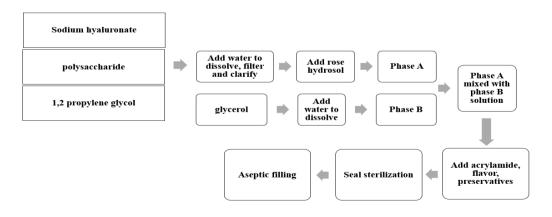


Fig. 1: The preparation process of essence liquid

The ethanol extraction method was used to extract flavonoids. According to previous research, the optimal extraction conditions were as follows: The dried stems of TC were ground and sieved, the material to liquid (ethanol solution 95%, v/v) ratio was 1:60 (g/mL), extract temperature 80°Cfor 3 (h). The extract was filtered and concentrated by evaporation (RE-5220A, Shanghai Zhengqiao Factory Scientific Instrument Co., Ltd.) and dried by vacuum freeze drier to obtain the crude flavonoid powder of TC stem.

#### Essence Liquid Preparation

Previous research had revealed that compared with flavonoids or polysaccharides from TC stem, the TC compound extract (TCCE), flavonoids and polysaccharides mixed in a mass ratio of 7:3, exhibited excellent antioxidantantioxidant activity and moisturizing performance, especially exhibited the highest hydroxyl radical scavenging (Zhang *et al.*, 2022). Hence, the flavonoids and polysaccharides compound of TC stem was used as the main ingredient.

In the context of cosmetic formulations, 1,2propanediol serves as an effective humectant, aiding in moisture retention and as a solvent, enhancing the homogeneity of product compositions.

Additionally, it is characterized by minimal skin irritation, which makes it widely utilized in the cosmetics industry (Yu et al., 2022). Sodium hyaluronate is another effective humectant in cosmetic formulations. Extensive research has confirmed that sodium hyaluronate comprises N-acetylglucosamine and D-glucuronic acid. It exhibits superior moisturizing and anti-aging efficacy even at low concentrations, approximately 0.1% (Nakajima et al., 2024). Glycerol is one of the most common primary humectants found in the majority of cosmetic formulations. Research has also demonstrated that glycerol when used at concentrations of 3-5%, exhibits excellent synergistic moisturizing effects in combination with other humectants such as 1,2propanediol and sodium hyaluronate. The inclusion of rose hydrosol in cosmetic formulations can offer multiple benefits: Its phenyl ethyl alcohol content provides a natural, pleasant aroma; flavonoids act as antioxidants that protect the skin from oxidative stress; and the mineral content supports overall skin health. These properties make rose hydrosol an advantageous ingredient for enhancing both the sensory appeal and functional efficacy of cosmetic products. In this research, optimizing the concentration of rose hydrosol will be crucial to achieving the desired effects on skin condition and product characteristics (Zhang et al., 2020). The unique characteristics of polyacrylamide enable it to increase the viscosity of aqueous solutions, thereby enhancing the texture and consistency of products such as creams, lotions and gels. Moreover, its suspending capability ensures the uniform dispersion of insoluble particles within the formulation, contributing to product stability over time. This makes polyacrylamide an indispensable ingredient for achieving desirable sensory attributes and performance in a wide range of cosmetic applications (Tanwar *et al.*, 2021).

As mentioned above, the basic formula of essence liquid was shown in Table (1) and the preparation process was as follows: Preparation of Phase A: Deionized water was heated to 80±5°C in a water bath (Shanghai Boxun Industrial Co., Ltd., HH. S11-1), sodium hyaluronate and the TCCE, mix the two solutions, were added in deionized water and stirred well, then rose hydrosol was added, at 80±5°C. Preparation of Phase B: Glycerol was added to dissolve flavonoids or polysaccharides from TC stormwater. Polyacrylamide was dissolved in water and was added to a mixture of phase A and phase to obtain a mixed emulsion. The mix solution was sealed and placed in a water bath at 80±5°C for 15 min for sterilization. After the preparation of the essence liquid, all the required experimental supplies were placed in the sterile operating platform for ultraviolet sterilization for 30 min, and then the essence liquid was canned in a sterile environment.

#### Evaluation of Moisturizing Activity of Essence Liquid

Moisture retention evaluations were conducted, drawing upon the study by Liu *et al.* (2015a) (Zhen *et al.*, 2024). To ensure consistent environmental conditions, the desiccator assembly was placed inside a constant temperature incubator maintained at 25°C. The essence liquid sample of 10.0 g was placed in a silica gel (43% RH) dryer for 12 h. Accurately weigh the mass with an analytical balance after being placed for 2, 4, 8, 10 and 12 h. The moisture retention activity of the samples was evaluated by the calculation formula as follows:

Moisture retention rate (%) = 
$$\left(\frac{H_1}{\mu}\right) \times 100\%$$
 (1)

where,  $H_0$  and  $H_1$  were the sample weights before and after being put in the desiccator, respectively.

#### Determination of Polyacrylamide Addition Amount

To enhance the stability of the essence liquid, polyacrylamide was added as an emulsifier. The addition amount was set as 0.7, 0.5, 0.3 and 0.1%. The addition amount was determined based on sensory evaluation (Chen *et al.*, 2015). The sensory evaluation score criteria were as follows Table (2).

Table 1: Ess	ence liquid for	mula of essen	ce liquid
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Table 1. Essence inquia formatia of essence inquia		
Component	Mass fraction (%)	
TCCE	1~5	
1,2-propanediol	2~6	
Sodium hyaluronate	0.1~0.5	
Glycerol	3~5	
Rose hydrosol	2~6	
Polyacrylamide	0.1~0.7	
Deionized water	make up to 100 mL	

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#### Table 2: Sensory evaluation score criteria

Standard	State	Score range	Score (Points)
Moderate viscosity	There is no wire drawing phenomenon, the viscosity is moderate, and there is no sedimentation after standing for a period of time	14-20	(= ====;)
Slightly viscous	There is a slight brushed phenomenon, slightly thick and smooth. After standing for a period of time, there is slight sedimentation	7-14	
Strong viscous	The drawing phenomenon is severe and relatively thick. When applied, it tends to be stagnant, and sedimentation is more obvious when left to stand	0-7	

## Single Factor Experimental Design for Essence Liquid

## Preparation of Essence Liquid

#### Determination of TCCE Addition Amount

Determination of the TCCE addition amount was designed as follows: The total mass of the essence liquid was set as 10.0 g, the addition amount of 1,2-propanediol 4%, sodium hyaluronate 0.3%, glycerol 4% and rose hydrosol 4%. The additional amount of TCCE was used as a variable, with 1, 2, 3, 4 and 5%, respectively. Then, the moisture retention rate of different group samples was determined.

#### Determination of 1,2-Propanediol Addition Amount

The additional amount of 1,2-propanediol was used as a variable, with 2, 3, 4, 5 and 6%, respectively (Chen *et al.*, 2020). The other steps were consistent with the previous description.

#### Determination of Sodium Hyaluronate Addition Amount

The additional amount of 1,2-propanediol was used as a variable, 0.1, 0.2, 0.3, 0.4 and 0.5%, respectively (Chen *et al.*, 2020). The other steps were consistent with the previous description.

#### Determination of Glycerol Addition Amount

The additional amount of glycerol as a variable, 3, 3.5, 4, 4.5 and 5%, respectively (Nie *et al.*, 2020). The other steps were consistent with the previous description.

#### Determination of Rose Hydrosol Addition Amount

The additional amount of rose hydrosol was used as a variable, with 2, 3, 4, 5 and 6%, respectively (Zhang *et al.*, 2020). The other steps were consistent with the previous description.

#### Orthogonal Experimental Design for Preparation of Tc Essence

Based on the single factor test, the  $L_{16}$  (4<sup>5</sup>) orthogonal experiment was conducted to optimize the best formula of essence liquid. The orthogonal experimental Table (3) was as follows.

#### Stability Evaluation of Essence Liquid

#### Heat and Cold Resistance Tests

Briefly, 10.0 g of essence liquid was taken into a test tube and sealed. Cold resistance test: The test tubes containing the essence liquid were stored under -40 °C for 24 (h), then they were taken out and observed for any layering, floating oil and color changes in the sample. Heat resistance test: The test tubes containing the essence liquid were stored under 80°C for 24 h; then they were taken out and observed for any stratification, oil slick and color changes of the sample.

#### Centrifugal Test

Briefly, 10.0 g of essence liquid was divided into two groups and put into 5 mL centrifuge tubes respectively. Under room temperature, the tubes were placed in a centrifuge and centrifuged at 1000, 2000, 3000, 4000, and 5000 r/min for 10 min. Then, they were taken out and observed for any stratification changes in the sample.

#### Appearance and Delicacy

The essence liquid solution with 10.0 g was placed in a 50 mL beaker, and its appearance was observed. The delicacy of the essence liquid was observed by applying it to the skin (Zhao *et al.*, 2008).

#### PH Test

The pH value of the essence liquid was tested by a pH meter (Ren *et al.*, 2021). The essence liquid solution with 10.0 g was dissolved in 100 mL of distilled water, then they were heated to a slight boiling point and then cooled to room temperature for measurement.

#### Skin Irritation Test

The measurement method was consistent with the relevant research (Li *et al.*, 2021). Volunteers of 30 were selected with healthy skin, including 10 aged 18-25, 10 aged 26-35 and 10 aged 36-45.

 Table 3: Orthogonal experiment of factors and levels

	А	В	С	D	Е
		1,2-		Sodium	
Level	TCCE	propanedi	Glycerol	hyaluron	Hydrosol
	(%)	ol	(%)	ate	(%)
		(%)		(%)	
1	1.0	4.5	4.0	0.4	3.0
2	1.5	5.0	4.5	0.5	4.0
3	2.0	5.5	5.0	0.6	5.0
4	2.5	6.0	5.5	0.7	6.0

The essence liquid of 0.5 g was smeared on the left back of the subject with an area of about 15 cm. The placebo testing was conducted alongside; the right back served as control. After 12 (h), the skin phenomenon was observed continuously for one week.

#### Skin Moisture Test

Volunteers of 10 subjects were selected with healthy skin for the moisture test. The subjects need to meet the following conditions: No serious systemic diseases, immunodeficiency or autoimmune diseases; no active allergic diseases; no history of severe allergies to skincare cosmetics; not using hormone drugs or the immunosuppressants in past month: not participating in other clinical trials. The test temperature was 22±1°C; the relative humidity was  $40\pm5\%$ . Before the test, the subjects should wipe their arms with water at around 35 °C and then enter the testing environment to rest for 30 min before starting the test. The testing process is divided into four parts: Before application, after application, 15 min after application, and after washing with water.

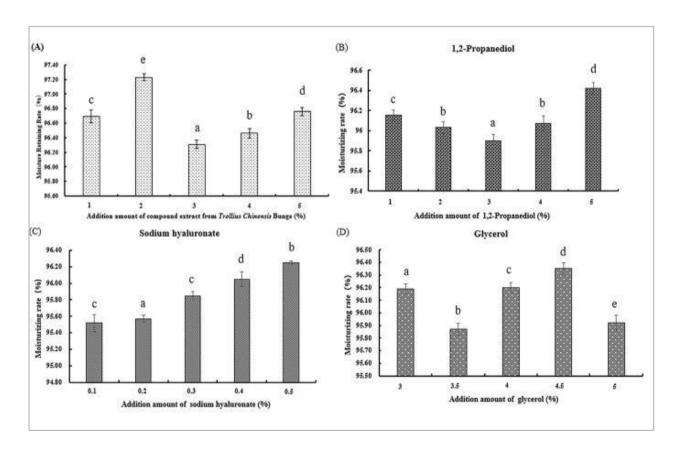
Note: A represents the effect of TCCE; B represents the effect of 1,2-propanediol; C represents the effect of sodium hyaluronate; D represents the effect of glycerol; E represents the effect of rose hydrosol.

#### **Results and Discussion**

#### Analysis of Single-Factor Experimental Results

On the basis of a single factor, the formula of essence liquid was optimized. The specific experimental results of the  $L_{16}$  (4<sup>5</sup>) orthogonal test are shown in Fig. (2).

From Fig. (2A), it can be seen that the highest moisturizing rate was achieved when the additional amount of TCCE was 2%. The moisturizing ability was related to the number of hydrophilic groups and the strength of hydrophilicity. The higher the concentration, the more hydroxyl groups it contains, which weakens the binding ability with hydrogen bonds and reduces the moisturizing rate (Sun et al., 2016). Studies have shown that the moisturizing rate will show a significant downward trend after the concentration exceeds 5%. In Fig. (2B), it can be observed that the moisturizing rate was the highest when the addition amount of 1.2-propanediol was 5%. When the additional amount of glycerol was 4.5%, the moisturizing rate reached its highest (Fig. 2C). From Fig. (2D), the moisturizing rate showed an increasing trend with the increased addition amount of sodium hyaluronate. When the addition amount was 0.5%, the moisturizing rate reached its highest. When the additional amount of rose hydrosol reached 4%, the moisturizing rate reached its highest Fig. (2E).



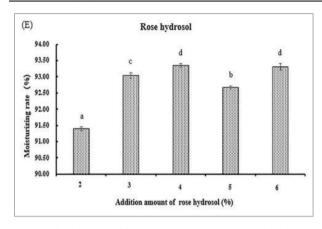


Fig. 2: The effect of different components on moisturizing rate

In summary, based on single-factor experiments, it can be preliminarily determined that the additional amount of the TCCE, 1,2-propanediol, glycerol, sodium hyaluronate and rose hydrosol were 2, 5, 4.5, 0.5 and 4%, respectively. The optimal formula of essence liquid was determined by an orthogonal test.

#### Determination of Polyacrylamide Addition Amount

After being evaluated by 6 trained sensory evaluators, the comprehensive score was calculated. According to the sensory evaluation score, when the additional amount of polyacrylamide was 0.3%, the essence liquid had better viscosity, ductility and stability. The results are shown in Fig. (3). The results depicted in Fig. (3) indicate that the 0.3% addition amount of polyacrylamide showed the highest score, while the 0.7% addition amount showed the lowest score. Polyacrylamide, widely recognized as an effective thickening and moisturizing agent in cosmetic formulations, has been shown in numerous studies to exhibit optimal efficacy within the low concentration range (Tanwar et al., 2021). Given these findings, it was determined that polyacrylamide does not require single-

factor optimization in this experiment, as its most suitable concentration can be confidently established at 0.3%. This concentration ensures that the product maintains its desired sensory attributes and functionality without compromising user satisfaction.

#### Analysis of Orthogonal Experiment Results

On the basis of a single factor, the formula of essence liquid was optimized. The specific experimental results of the  $L_{16}$  (4<sup>5</sup>) orthogonal test are shown in Table (4). According to the orthogonal experiment results in Table (4), the optimal combination was  $A_4B_4C_1D_3E_2$ . Specifically, the additional amount of the TCCE, 1,2-propanediol, glycerol, sodium hyaluronate and rose hydrosol were 2.5, 6.0, 4.0, 0.6 and 4.0%, respectively. The moisturizing rate was up to 99.30%. The Range value (R) order of different factors was as follows: R<sub>D</sub>>R<sub>C</sub>>R<sub>B</sub>>R<sub>E</sub>>R<sub>A</sub>. Therefore, among the factors set in the experiment, sodium hyaluronate had the greatest impact on the moisture retention rate of essence liquid, followed by glycerol, 1,2propanediol, rose hydrosol, and TCCE.

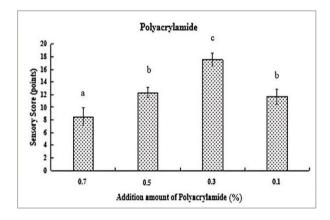


Fig. 3: Sensory score of different polyacrylamide addition amount

Table 4: Orthogonal experiment results						
Factor test	A (%)	B (%)	C (%)	D (%)	E (%)	Moisturizing rate (%)
1	1.0	4.5	4.0	0.4	3.0	98.82
2	1.0	5.0	4.5	0.5	4.0	99.13
3	1.0	5.5	5.0	0.6	5.0	98.73
4	1.0	6.0	5.5	0.7	6.0	98.93
5	1.5	4.5	4.5	0.6	6.0	99.13
6	1.5	5.0	4.0	0.7	5.0	98.94
7	1.5	5.5	5.5	0.4	4.0	98.88
8	1.5	6.0	5.0	0.5	3.0	99.01
9	2.0	4.5	5.0	0.7	4.0	98.80
10	2.0	5.0	4.0	0.6	3.0	99.06
11	2.0	5.5	5.5	0.5	6.0	99.02
12	2.0	6.0	4.5	0.4	5.0	99.03
13	2.5	4.5	5.5	0.5	5.0	99.26
14	2.5	5.0	5.0	0.4	6.0	98.99
15	2.5	5.5	4.5	0.7	3.0	98.94
16	2.5	6.0	4.0	0.6	4.0	99.30
R	0.0012	0.0014	0.0018	0.0020	0.0013	

#### Stability Evaluation Results of Essence Liquid

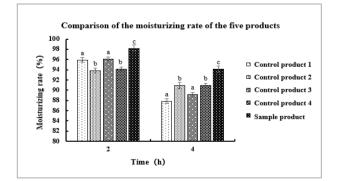
The stability results of the product after optimizing the optimal formula are shown in Table (5). The essence liquid possessed good cold and heat resistance characteristics, without layering, with a light brown color and a faint rose aroma. The essence liquid was relatively uniform, and the texture was delicate. The pH of the product was close to neutral, and it was not irritating to the skin.

#### Moisturizing Rate Comparison of Essence Liquid

The moisturizing rate of essence liquid was compared with four other popular commercial product products. After 2-4 (h), the moisturizing rates of the five products were measured and compared. The results are shown in Fig. (4).

 Table 5: Stability and performance evaluation results of essence liquid

Project	Evaluation results
Cold-resistant	After 24 (h), there was no significant change at room temperature
Heat-resisting	After 24 (h), there was no significant change at room temperature
Centrifugal test	No <sup>stratification</sup> occurred after centrifugation
Appearance and delicacy	The color is light brown with a faint rose aroma. The finished product is relatively uniform, and the texture is delicate
pH value	7.27±0.52
Viscosity	1.25±0.08 Pa·s
Skin irritation test	No allergic reactions



**Fig. 4:** Comparison of moisturizing rates of essence liquid Note: Lowercase letters indicated significant differences between groups (p<0.05). A: Control product 1, B: Control product 2, C: Control product 3, D: Control product 4, E: Sample product 1

In Fig. (4), it can be seen that Product 1 and Product 3 had better effects when first used, but the moisturizing rate decreased rapidly. Although the moisturizing rate of products 2-4 was lower than that of Products 1-3 in the early stage, the decrease in moisturizing rate was slower, and the moisturizing rate was relatively higher after 4 (h). The moisturizing rate of the essence liquid sample in the early stage was higher than the other four products, and the moisturizing rate after 4 (h) was also higher than the other four products. The results presented in Fig. (4) indicate that the sample product, referred to as the optimized essence liquid. exhibits superior moisturizing effects compared to other control products at both 2-4 (h) post-application. This enhanced efficacy can be attributed to two potential factors. Firstly, the extract from the stem of Trollius chinensis may inherently possess excellent moisturizing properties (Zhang et al., 2022). Secondly, the synergistic action among the chosen humectants, such as glycerol, 1,2propanediol and sodium hyaluronate, would optimize moisture retention within the stratum corneum, leading to improved skin hydration levels over time. The combination of these factors likely contributes to the superior performance of the sample product in maintaining skin hydration (Qian et al., 2022).

Among the other four products, Products 2 and 4 have similar moisturizing effects, slightly higher than products 1 and 3. Finally, product 4 product was selected as the control product for the skin moisture test.

#### Skin Moisture Test Comparison of Essence Liquid

A skin moisture test comparison was carried out by the skin moisture tester (CM825-MDD, Beijing Jinhong Trading Co., Ltd.). The measurement method was based on the research (Fang *et al.*, 2015; Liu *et al.*, 2015b) and had been modified accordingly. The test results are shown in Fig. (5).

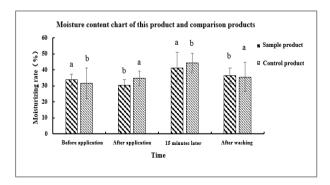


Fig. 5: Skin moisture test comparison between essence liquid and control product

According to Fig. (5), it can be seen that there was no significant difference between the essence liquid and control product before application, after application, 15 min after application and washing. Compared to the control products, the sample product demonstrated superior moisturizing performance at all tested stages: Before and after application and after washing. This consistent and good moisturizing effect confirms the excellent hydrating properties of the developed formulation. The control product is one of the higher-priced, well-selling products in the Chinese market, known for its efficacy and consumer acceptance. The essence liquid developed by this research, one of the main raw materials in the formula, comes from the waste of natural plants, which has the advantages of low cost and excellent moisturizing effect compared with the control product. If approved for production at a later stage, it will have a strong competitive advantage.

## Conclusion

This experiment developed the essence liquid containing the flavonoids and polysaccharides compound from the TC waste stem. Through the single factor experiment and  $L_{16}$  (4<sup>5</sup>) orthogonal experiment, the formula of the essence liquid was finally determined as follows: TCCE 2.5%, 1,2-propanediol 6%, glycerol 4%, sodium hyaluronate 0.6%, rose hydrosol 4%. The moisturizing rate of the optimized essence liquid was up to 99.3%. The optimized essence liquid possessed cold and heat resistance and good stability without any layering. The pH was 7.27, which was weakly alkaline and safe to use. Compared with the four control products, the optimized essence liquid exhibited an advantage in the moisture retention rate test. In the skin moisture test comparison, there was no significant difference between the essence liquid and control product before application, after application, 15 min after application and after washing. This study provided certain theoretical value for the development and utilization of waste stemming from TC. This will have a profound impact on the high-value recycling and utilization of Trollius chinensis Bunge byproducts, as well as the improvement of comprehensive utilization and commercialization levels.

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## **Author's Contributions**

**Xiyue Yu:** Participated in the whole experiment and also contributed to the interpretation of the results, manuscript preparation, and written of the original draft.

Jiaqing Wu and Wenqiu Zhu: Participated in the experimental design.

Bing Li: Data collection and processing.

Lanlan Zhang and Guangxin Tan: Data collection.

Yang Liu: Conceptualization, Funding acquisition, Written-review and edited.

## **Ethics**

All the authors have read and approved the manuscript, and no ethical issues involved. This article is original and contains unpublished material.

## References

- Agregán, R., Pateiro, M., Bohrer, B. M., Shariati, M. A., Nawaz, A., Gohari, G., & Lorenzo, J. M. (2023). Biological Activity and Development of Functional Foods Fortified with Okra (*Abelmoschus Esculentus*). Critical Reviews in Food Science and Nutrition, 63(23), 6018-6033. https://doi.org/10.1080/10408398.2022.2026874
- Chen, J. L., Zhang, Y. Q., Yuan, Y., Wu, S. R., & Ming, J. (2015). Progress in Research on Immune-Regulatory Effects of Plant Polysaccharides on Macrophages through NF-κB Signaling Pathway. In *Food Science* (Vol. 36, Issue 23, pp. 288-294).
- Chen, S., Niu, L. C., Wang, X. Y., Lu, F., Nie, Y. Y., Li, Y. Z., Wang, J. F., & Li, B. (2020). Development of Essence Liquid of Pleurotus Eryngii Facial Mask. In *Edible fungi* (Vol. 42, Issue 04, pp. 65-67).
- Fang, Y. J., Xia, D. Z. W., S.W., & Ji, S. S. (2015). Study on Anti-Obese and Hypolipidemic Effects of Total Flavonoids from Alpinia Officinarum Hance in Rats with Nutritive Obesity Combined with Hyperlipidemia. In *China Journal of Traditional Chinese Medicine and Pharmacy* (Vol. 30, Issue 08, pp. 2907-2910).
- Fournière, M., Bedoux, G., Lebonvallet, N., Leschiera, R., Le Goff-Pain, C., Bourgougnon, N., & Latire, T. (2021). Poly- and Oligosaccharide Ulva sp. Fractions from Enzyme-Assisted Extraction Modulate the Metabolism of Extracellular Matrix in Human Skin Fibroblasts: Potential in Anti-Aging Dermo-Cosmetic Applications. *Marine Drugs*, 19(3), 156. https://doi.org/10.3390/md19030156

Habtemariam, S. (1997). Flavonoids as Inhibitors or Enhancers of the Cytotoxicity of Tumor Necrosis Factor-α in L-929 Tumor Cells. *Journal of Natural Products*, 60(8), 775-778.

https://doi.org/10.1021/np960581z

- Hu, C.-Q., Chen, K., Shi, Q., Kilkuskie, R. E., Cheng, Y.-C., & Lee, K.-H. (1994). Anti-AIDS Agents, 10. Acacetin-7-O-β-D-Galactopyranoside, An Anti-HIV Principle from Chrysanthemum Morifolium and a Structure-Activity Correlation with Some Related Flavonoids. *Journal of Natural Products*, 57(1), 42-51. https://doi.org/10.1021/np50103a006
- Hollman, P. C. H., & Katan, M. B. (1999). Dietary Flavonoids: Intake, Health Effects and Bioavailability. *Food and Chemical Toxicology*, *37*(9–10), 937-942.

https://doi.org/10.1016/s0278-6915(99)00079-4

- Lee, B. C., Bae, J. T., Pyo, H. B., Choe, T. B., Kim, S. W., Hwang, H. J., & Yun, J. W. (2003). Biological Activities of the Polysaccharides Produced from Submerged Culture of the Edible Basidiomycete Grifola Frondosa. *Enzyme and Microbial Technology*, 32(5), 574-581. https://doi.org/10.1016/s0141-0229(03)00026-7
- Li, B., Zhou, Y. L., Zhang, Y. R., Lu, S., Zhang, P. H., Cui, Y. J., Chen, C. C., & Li, Y. Z. (2021). Preparation of Ganoderma Lucidum Facial Mask Essence Liquid. In *Edible fungi* (Vol. 43, Issue 06, pp. 71-74).
- Li, C. (2017). Preparation and Film-Forming Properties of Nano Polysaccharide Particles from Flammulina Velutipes (pp. 3-25).
- Li, Q., Zhang, J. J., He, Q. J., & Zhen, W. H. (2015). Preliminary Study on the Moisture Absorption and Moisturizing Properties of Brown Algae Polysaccharide Sulfate Derivatives. In *Strait Pharmaceutical Journa* (Vol. 27, Issue 05, pp. 40-41).
- Liu, G., Yang, X., Zhang, J., Liang, L., Miao, F., Ji, T., Ye, Z., Chu, M., Ren, J., & Xu, X. (2021). Synthesis, Stability and Anti-Fatigue Activity of Selenium Nanoparticles Stabilized by Lycium Barbarum Polysaccharides. *International Journal of Biological Macromolecules*, 179, 418-428.

https://doi.org/10.1016/j.ijbiomac.2021.03.018

Liu, J., Willför, S., & Xu, C. (2015a). A Review of Bioactive Plant Polysaccharides: Biological Activities, Functionalization and Biomedical Applications. *Bioactive Carbohydrates and Dietary Fibre*, 5(1), 31-61.

https://doi.org/10.1016/j.bcdf.2014.12.001

Liu, X., Liu, F., Liu, S. Y., Chen, X. Y., Meng, X. J., & Zhu, X. Q. (2015b). Moisture Retention Capacity and Safety Evaluation of Poly-γ-Glutamate. In *China Surfactant Detergent & Cosmetics* (Vol. 45, Issue 05, pp. 275-278).

- Liu, Y., Guo, Y. C., Cai, L. J., & Long, X. Y. (2018). Preparation and Performance Evaluation of Lycium Barbarum Polysaccharide Mask. In *China Surfactant Detergent and Cosmetics* (Vol. 48, Issue 11, pp. 39-44).
- Liu, Y., Zhou, H. L., Ma, X. H., Deng, M. J., Qin, M. M., & Sui, X. (2017). Total Flavonoids Antioxidation Activity of Trollius chinensis Bunge in Different Parts. *Food Research and Development*, 38(14), 7-11.
- Liu, Y., Zhou, H. L., Ma, X. H., Li, W. H., Chi, G. X., & Sui, X. (2016). Purification Technology Optimization of Total Flavonoids from Trollius Chinensis Bunge in Daxing'an Mountain. *Food Tchnology*, 41(12), 163-168.
- Luo, H., Tan, D., Peng, B., Zhang, S., Vong, C. T., Yang, Z., Wang, Y., & Lin, Z. (2022). The Pharmacological Rationales and Molecular Mechanisms of *Ganoderma lucidum*Polysaccharides for the Therapeutic Applications of Multiple Diseases. *The American Journal of Chinese Medicine*, 50(01), 53-90. https://doi.org/10.1142/s0192415x22500033
- Ma, S., Zhou, J.-M., Wei, H.-S., & Wu, H.-B. (2020). Flavones from the Flowers of Tridax Procumbens and their AntioxidantAntioxidant Activity. *Chemistry of Natural Compounds*, *56*(2), 239-241. https://doi.org/10.1007/s10600-020-02996-2
- Nakajima, K., Tran Vo, T. M., & Adlin, N. (2024). Utilization of Multi-Ionic Interaction of Yumoto Hot Springs for Enhancing the Moisturizing Properties of Hyaluronic Acid Sodium Salt. *Polysaccharides*, 5(2), 100-111.
- https://doi.org/10.3390/polysaccharides5020008
  Nie, Y. Y., Wang, X. Y., Zhang, X. N., Li, Y. Z., Cao, X. Y., & Li, B. (2020). Preparation of Facial Mask Essence Adding Flammulina Velutipes. *Journal of Henan Institute of Science and Technology (Natural*)
- Science Edition, 48(06), 43-47.
  Oliveira, A. P., Silva, A. L. N., Viana, L. G. F. C., Silva, M. G., Lavor, É. M., Oliveira-Júnior, R. G., Alencar-Filho, E. B., Lima, R. S., Mendes, R. L., Rolim, L. A., Anjos, D. S. C., Ferraz, L. R. M., Rolim-Neto, P. J., Silva, M. F. S., Pessoa, C. do Ó., & Almeida, J. R. G. S. (2019). β-Cyclodextrin Complex Improves the Bioavailability and Antitumor Potential of Cirsiliol, A Flavone Isolated from Leonotis Nepetifolia (Lamiaceae). *Heliyon*, 5(10), e01692. https://doi.org/10.1016/j.heliyon.2019.e01692
- Patel, K. B., & Kumari, P. (2022). Anticancer Activity and Docking Study of Flavone Derivatives as Peroxisome Proliferator-Activated Receptorγ Inhibitors. *Structural Chemistry*, 33(6), 1835-1851. https://doi.org/10.1007/s11224-022-01926-y
- Qian, J., Deng, H., Li, H., & Li, C. (2022). Research on Antagonistic and Synergistic Effects of Moisturizers in Cosmetics. *Guangdong Chemical Industry*, 49(19), 49-54.

- Ren, Y., Zhang, X. Q., Wang, H. B., Chai, L. Z., H.Q., Y., & Dong, S. T. (2021). Preparation and Efficacy Evaluation of Dendrobium Devoninum Mask. *Yunnan Chemical Technology*, 48(03), 23-28.
- Robert, C., Robert, A. M., & Robert, L. (2003). Effect of a Fucose-Rich Polysaccharide Preparation on the Age-Dependent Evolution of the Skin Surface Micro-Relief. *Pathologie Biologie*, 51(10), 586–590. https://doi.org/10.1016/j.patbio.2003.09.009
- Sebti, I., & Coma, V. (2002). Active Edible Polysaccharide Coating and Interactions Between Solution Coating Compounds. *Carbohydrate Polymers*, 49(2), 139-144.

https://doi.org/10.1016/s0144-8617(01)00315-0

Shan, C., Shu, C., Nie, W., Xu, Y., Ma, S., & Hu, Q. (2022). RETRACTED ARTICLE: Anti-Aging Effect of Polysaccharides from the Peel of Zanthoxylum schinifolium Sieb. et Zucc. on the Nematode Caenorhabditis Elegans Acta Agriculturae Scandinavica, Section B Soil & Plant Science, 72(1), 476–484.

https://doi.org/10.1080/09064710.2021.2012249

- Sun, F. Y., Wang, M., Wang, J. Z., Hao, H. X. Y., H.S., & Qiao, C. S. (2016). Viscosity Stability and Moisturizing Performance of Pullulan.
- Tanwar, A., Date, P., & Ottoor, D. (2021). ZnO NPs Incorporated Gelatin Grafted Polyacrylamide Hydrogel Nanocomposite for Controlled Release of Ciprofloxacin. Colloid and Interface Science Communications, 42, 100413. https://doi.org/10.1016/j.eclopm.2021.100412

https://doi.org/10.1016/j.colcom.2021.100413

Wang, L., Tian, Y., Zhang, P., Li, C., & Chen, J. (2022). Polysaccharide Isolated from Rosa roxburghii Tratt Fruit as a Stabilizing and Reducing Agent for the Synthesis of Silver Nanoparticles: Antibacterial and Preservative Properties. *Journal of Food Measurement* and Characterization, 16(2), 1241–1251. https://doi.org/10.1007/s11694-021-01248-3

- Wang, S., Rao, N., & An, F. (2013). Purification and AntioxidantAntioxidant Activity of Polysaccharide Isolated from Trolliouschinensis. *Chinese Traditional Patent Medicine*, 35(11), 2384–2389.
- Wang, W., Wu, M., Si, M. D., & Ma, D. L. (2020). Quality Evaluation of Trollius Chinensis Bunge from Different Originsbasedon Principal Component Analysis. *Fine* and Specialty Chemicals, 28(11), 32–39.
- Yu, C., Yao, Q., Tang, J., & Zhu, Y. (2022). Study on Hygroscopic and Moisturizing Properties of Common Diols. *Guangdong Chemical Industry*, 49(23), 58–60.
- Yu, L., Zhang, J., Jiao, J., Su, J., Sun, W. T., Guo, Y., Ma, S. X., Zhang, T., & Meng, D. X. (2020). Effect of Nano yam Polysaccharide on the Blood Glucose and Blood Lipid in Rats. *Pakistan Journal of Pharmaceutical Sciences*, 33(1), 481–487.
- Zhao, L., Liu, W., & H.Y, W. (2008). Gener al Guidance and Method for Establishing Index System of Food Sensor Y Evaluation. *Journal of Chinese Institute of Food Science and Technology*, 03, 121–124.
- Zhang, H. G., Zhang, X. B., Hu, Y., Shi, X. X., & Wang, J. (2020). Preparation of Rose Hydrolat and Its Application in Cosmetics. *Guangdong Chemical Industry*, 47(18), 103–104.
- Zhang, S., Guo, Q., Xin, Y., Li, Q. X., & Liu, Y. (2022). Synergistic Moisturizing Activity and AntioxidantAntioxidant Activity Between Crude Polysaccharides and Flavonoids in Discarded Stem of Trollius Chinensis Bunge. *Engeering Report*, 5, 12569.
- Zhen, Y., Hu, X. X. & Z., & L.O. (2024). Standard Test Guidelines for Skin Safety and Efficacy Testing of Cosmetics. Asian Journal of Ecotoxicology, 19(04), 88–99.
- Zhao, S. (2019). Studies on Skin Care Characteristics and White Ningmechanism of the Composite of Silk Fibroin Oligopeptide with Cordyceps Militaris polysaccharide.