Poly Traditional Chinese Medicine formulation prepared with skin moisturizing properties

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Research

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Abstract

Background

Many traditional Chinese medicine compositions can moisturize the skin and utilize in cosmetics. Using a combination of Chinese Medicine Materials and guided by Traditional Chinese Medicine principles, this study selected Echinacea purpurea to protect the skin barrier, Dendrobium nobile to clear heat and promote fluid production, Sophora flavescens to clear heat for diminished inflammation, and Aloe vera combined Lycium barbarum to nourish yin, to together form a "poly TCM moisturizing formulation".

Methods

These poly plant extracts were investigated and optimized for the stability, safety and moisturizing ability. The combination moisturizing effect was determined by measuring the expression of FLG mRNA, CLDN-1 mRNA and AQP3 protein. Toxicological analysis included a red blood cell hemolysis test and a 3T3 phototoxicity test .

Results

It has been observed that by using polysaccharide yield as the evaluation criterion showed optimal extraction at a material-to-liquid ratio of 1:100, an extraction temperature of 100 °C, and an extraction time of 3 h. Moisturizing effect experiments showed that the expression of FLG mRNA, CLDN-1 mRNA and AQP3 protein was significantly increased. Toxicological tests showed that the composition was safe and caused no irritating effects.

Conclusion

Based on these results, this poly TCM moisturizing formulation is safe within moisturizing effects and can be used as a moisturizing raw material in cosmetics.

1 Backgroud

Dry skin is an uncomfortable condition marked by scaling, itching, and cracking. Dry skin is caused by a decrease in the moisture content of the skin and an increase in water dispersion. Losses due to water dispersion has a greater effect than the moisture content on whether the skin is dry. At the same time, skin water loss is an important indicator of the quality of the skin barrier, so dry skin may be caused by damage to the skin barrier. Dry skin can cause scaling and fine lines. Dry skin caused by impaired skin immune barrier function accelerates skin aging[1]. At present, hydration alone is usually used to relieve dryness in dry skin, but it cannot fundamentally solve the problem.

Recently, the hygienic field of cosmetics and the pursuit of natural consumer crazes have prompted domestic and foreign cosmetics companies to pay increasing attention to the research and development of cosmetic plant compositions.

As the original medical science of the Chinese nation, traditional Chinese medicine (TCM) can reveal the laws of human health and disease occurrence from a macroscopic, systematic and holistic perspective and embody the cognitive style of the Chinese nation, and it has been deeply integrated into the productivity and lives of people, forming a unique health culture and practice. As the core elements of the basic process of TCM diagnosis and problem solving, "symptom, reason, method, prescription, medicine, efficacy" is an approach that is applied consistently throughout the research and application of skin care products[2].

In accordance with the principle of “monarch, minister, assistant and guide” in Chinese medicine, *Echinacea purpurea* Linn. solid water protects the skin barrier (monarch), *Dendrobium nobile* Lindl. clear heats and promotes fluid production (minister), *Sophora flavescens* Ait. clears heat to reduce inflammation (assistant), and *Aloe vera* L. and *Lycium barbarum* L. nourish body's essential fluid to together form a "plant moisturizing composition"

E. purpurea, with a solid water barrier, is selected as the monarch. *Echinacea* is an excellent immune promoter and immunomodulator, and its abundant polysaccharide components (such as 4-methoxy-glucaldehyde-arabinose-xylan) exhibit significant enhancement of humoral immune function[3]. It is used as a treatment for trauma, eczema, etc., and it improves skin barrier function and skin immunity[4]. The plant moisturizing composition uses purple Echinacea as the monarch and uses it to retain water to protect the skin barrier.

D. nobile is commonly known as “millennial nourishment” and is a traditional rehydration herb. Modern studies have shown that *D. nobile* polysaccharide not only has an exogenous moisturizing effect but also promotes the expression of aquaporin 3 in epidermal keratinocytes[5]. Therefore, the plant moisturizing composition takes *D. nobile* as the minister and uses its rehydration and fluid effects.

From the perspective of skin physiology, inflammatory factors and other "hot poisons" inhibit the endogenous water transport of the skin, causing endogenous water to be transported to the stratum corneum of the skin surface and thus making the skin dry and dehydrated. *S. flavescens* is a traditional heat-clearing medicine. According to pharmacology, *S. flavescens* has the effect of "heat-treating toxic wind, skin muscle irritating sores"[6]. Therefore, the plant moisturizing composition here is based on bitter ginseng, which clears heat and reduces inflammation[7].

A. barbadensis has been a skin care product since ancient times. Some amino acids and metal salts contained in *A. vera* are the same as the natural moisturizing factors contained in human skin, making its moisturizing properties prominent[8]. Polysaccharides that are abundant in *A. vera* have strong penetrating ability and are easily absorbed by the skin to accomplish its moisturization. *A. vera* is listed in the " Shennong's Classic of Materia Medica " as a top grade component. The "Compendium of Materia Medica" states that sweet in flavor and mildly moist in property and is a good product for nourishing yin and health. *L. barbarum* is rich in nutrients such as carotene, vitamin A, calcium and iron. It can activate skin cells, promote cell metabolism, improve skin's water retention, and help the skin remain full, smooth and delicate[9]. The plant moisturizing composition here includes *A. vera* and *L. barbarum*.

P. Echinacea solid water barrier in the prescription can solve the problem of barrier repair in dry skin, reducing skin water loss[10]. *D. nobile* can replenish skin moisture and promote endogenous water production and transport. *S. flavescens* can eliminate inflammatory factors that inhibit endogenous water transport in skin. *A. vera* and *L. barbarum* maintain the skin oil-water balance and nutritional balance by nourishing yin.

This investigation found that the plant composition has a good moisturizing effect. This research will study the extraction process, moisturizing effect and safety of this plant moisturizing composition, aiming to develop new raw materials to increase the moisturizing efficacy of cosmetics.

2 Material And Methods

2.1 Chemical and reagents

Keratinocyte culture medium (4th Military Medical University Tissue Engineering Center, Xi'An, China), PBS (Boster,Hubei, China), MTT (Sigma, Darmstadt, Germany), DMSO (Sigma, Darmstadt, Germany), RNA extraction kit (TaKaRa Bio, Tokyo, Japan), reverse transcription kit (TaKaRa Bio, Tokyo, Japan), fluorescent dye (TaKaRa Bio, Tokyo, Japan), AQP3 antibody (Santa, CA, USA), fluorescent secondary antibody (Santa, CA, USA), blocked sheep serum (Zsbio, Beijing, China), and antifluorescence quenching and sealing liquid (Beyotime, Shanghai, China) were used.

A CO_2 incubator (Themo, MA, USA), an ultraclean workbench, an inverted microscope (Olympus, Tokyo, Japan), a micro-oscillator (Qilinbeier, Jiangsu, China), a microplate reader (BioTek, VT, USA), a real-time PCR instrument (Bio-Rad, CA, USA), and a laser confocal microscope (Olympus, Tokyo, Japan) were also used.

2.2 Plant Material Collection And Extraction Process Optimization

In this study, the whole grass of *E. purpurea*, the stems of *D. nobile*, the leaves of aloe, the roots of *S. flavescens*, and the fruits of Chinese wolfberry were smashed through a 6 mesh sieve. The effects of extraction temperature, solid-liquid ratio and extraction time on polysaccharide content were investigated by 3-factor and 3-level orthogonal tests.

2.3 Preliminary Qualitative Phytochemical Analysis Of Plant Extracts

A large number of reports have stated that plant polysaccharides have good moisturizing and skin care effects. To determine whether polysaccharides were present in the plant extracts, aqueous plant extracts were prepared for testing.

2.4 Test For Polysaccharide

Take 1 g of sample, add 1 ml 15% TCA solution, add a little 5% TCA solution, pour the supernatant into a 10 ml centrifuge tube, add a little 5% TCA solution to grind, pour the supernatant, and repeat 3 times. Centrifuge at 3,000 rpm for three times. After adding 2 ml of 6 mol / L hydrochloric acid to the colorimetric tube, shake well, and then water-bath in a 96 ° C water bath for 2 hours. After the water bath, cool with running water and add 2 ml of 6 mol / L sodium hydroxide to shake. Make up to a 25 ml volumetric flask. Pipette 0.2 ml of the sample solution, make up to 2.0 ml by distillation, then add 1.0 ml of 6% phenol and 5.0 ml of concentrated sulfuric acid, shake and cool for 20 minutes at room temperature and measure the optical density at 490 nm. Take two samples for each measurement. The polysaccharide content was calculated using a standard curve. The total polysaccharide content was detected[10–13].

2.5 Stability Investigation

The cosmetic plant composition was required to be strictly stable. The various conditions for testing the stability of a stock solution included room temperature (placed on the laboratory table top), protected from light (placed in a 28 ± 1 °C incubator cassette), illumination (in a 28 ± 1 °C constant temperature incubator placed under light), heat (placed in an oven at 45 ± 1 °C), alternating hot and cold (that is, a 28 ± 1 °C and 4 ± 1 °C alternating hot and cold incubator), refrigeration (placed in a 4 ± 1 °C refrigerator fresh-keeping layer), and freezing (placed in a -20 ± 1 °C freezer layer)[14]..

2.6 Cell Culture Of Keratinocytes

Keratinocytes were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) in a humidified atmosphere with 5% CO_2 at 37 °C. The medium was replaced every two days.

2.7 MTT Detection Of Keratinocytes

This test uses the MTT cell activity assay to screen for the maximum safe dose of the sample in keratinocytes. To ensure the validity of the MTT test results, 4% DMSO was used as a positive control (PC). The sample was set up with a gradient of 8 concentrations, and 3 replicate wells were set for each concentration. A solvent control (SC) and a zero well (cell culture) were set up[15].

2.8 Detection Of FLG And CLDN-1 Cell Immunofluorescence

Cells were seeded in 6-well plates and incubated overnight at 37 °C in a CO_2 incubator. The sample was diluted with DMSO to prepare a plant moisturizing composition at 125 ppm, 250 ppm, and 500 ppm. When the plating rate of the 6-well plate reached 40%-50%, group administration was performed, and each group was provided in 3 duplicate wells. A blank control group (1‰ DMSO) was added to the keratinocyte

culture medium, and the sample group was added to keratinocyte culture medium containing the corresponding concentration of the test substance. Culture was continued for 24 h at 37 °C in a 5% CO_2 incubator. The culture solution was discarded, 1 ml of TRIzol was added per well, the lysed cells were pipetted, and then a sample was taken. RNA was extracted and reverse transcribed into cDNA, and then quantitative PCR detection was performed.

2.9 Detection Of QP3 Cell Immunofluorescence

Cells were seeded in 24-well plates and incubated overnight at 37 °C in a CO_2 incubator. The sample was diluted with DMSO to prepare a plant moisturizing composition at 125 ppm, 250 ppm, and 500 ppm. When the cell plating rate in the 24-well plate reached 40%-50%, group administration was performed, and three duplicate wells were set in groups. The blank control group was supplemented with keratinocyte culture medium containing 1‰ DMSO, the sample group was supplemented with keratinocyte culture medium containing the corresponding concentration of the test substance, and culture was continued for 24 h at 37 °C in a 5% CO_2 incubator. After incubation, the medium in the 24-well plate was discarded, and the slides were washed three times with PBS. The cells were fixed with 4% paraformaldehyde at room temperature for 30 min and stored in a refrigerator at 4 °C. Cells were permeabilized, blocked with goat serum blocking solution at 37 °C for 30 min, incubated with primary antibody solution at 4 °C overnight, and incubated with secondary antibody solution at 37 °C for 2 h before the cell nuclei were dyed with Hoechst dye. (The infected nuclear region can be excited by blue light to emit blue fluorescence.) A photo was taken under a fluorescence microscope. Green fluorescence of the target protein was excited by the blue light channel in the same field of view, and the blue fluorescence of the nuclear region was excited by ultraviolet light and photographed separately.

2.10 Erythrocyte Hemolysis Test

Stimulation of the red blood cell membrane broke the red blood cell membrane, causing a certain degree of hemolysis. The absorbance of the red blood cell suspension after the action of chemical substances was measured by spectrophotometry, and the hemolysis rate was calculated. Among the tested chemicals, 0.02% sodium dodecyl sulfate (SDS) is potent as a PC for this test[16].

2.11 Sample Preparation For The 3T3 Neutral Red Test

The plant moisturizing composition was formulated into solutions of 8 concentrations (7.8, 15.6, 31.3, 62.5, 125.0, 250.0, 500.0 and 1000.0 μg/mL) with Hank's balanced salt solution (HBSS). Chlorpromazine (CPZ) hydrochloride was dissolved in absolute ethanol to prepare the highest-concentration mother liquor (20.00 mg/mL), which was then diluted to different concentrations with 50% absolute ethanol. The concentration was 0.02 mg/mL, and each solution was diluted 100 times with HBSS before use and standby application.

2.12 Cell Inoculation For The 3T3 Neutral Red Test

Well-grown Balb/c3T3 cells were inoculated in the wells of a 96-well culture plate (except for the peripheral wells) according to the guidelines of OECD432 so that the number of cells per well is 1.0×10^4, and then the plate was placed at 37 ± 1 °C and 5 ± 1% CO_2 for approximately 24 h to humidify the culture.

2.13 Dosing And Lighting For The 3T3 Neutral Red Test

After culturing of Balb/c 3T3 cells for 24 h, the culture solution was removed, and the cells were gently washed twice with 150 μL of prewarmed HBSS. Then, 100 μL of each test substance at different concentrations was added to each well. The control substance was HBSS, and there were 6 duplicate wells per concentration and 2 plates for each test substance and the PC, which were labeled “light plate” and “no light plate”, respectively. The culture plates were incubated at 37 ± 1 °C and 5 ± 1% CO_2 for 1 h, and the light plates were irradiated for 45 min under UVA with a light intensity of 1.7 ± 0.2 mw/cm^2 until the light dose reached 5.0 J/cm^2. The no-light plate was wrapped with tin foil and incubated with the light plate for the same time in a dark room at room temperature. After the end of the light treatment, the HBSS containing the test substance/control substance was removed, and the cells were gently washed twice with 150 μL of preheated HBSS per well. Then, the HBSS was replaced with fresh 10% (v/v) FBS and DMEM containing 4 mM glutamine, 100 IU penicillin and 100 μg/mL streptomycin, and the cells were cultured at 37 ± 1 °C and 5 ± 1% CO_2 for 18–22 h.

2.14 3T3 Neutral Red Test

Approximately 3 h before the end of culture, the medium of each well was discarded, the cells were gently washed once with 150 μL of prewarmed HBSS per well, and then 100 μL of 50.0 μg/mL neutral red was added serum-free per well. DMEM was cultured for 3 h at 37 ± 1 °C and 5 ± 1% CO_2. After the completion of the culture, the medium containing neutral red was discarded, and the cells were washed with 150 μL of preheated HBSS. Then, 150 μL of neutral red eluate was added to each well (water:ethanol:acetic acid = 49:50:1, v/v/v), an oscillator rapidly shook the sample until a homogeneous solution was formed, and then the absorbance (OD540) of each well was measured with a spectrophotometer at 540 nm[17].

2.15 Statistical analysis

Based on the measured absorbance values (OD540), Phototox Version 2.0 software was used to calculate the photo irritation factor (PIF) and mean photo effect (MPE) of each test substance. Under light and no-light conditions, the OD540 was measured for each well. The IC50, PIF and MPE of the test samples and the PC under light and no-light conditions were calculated using Phototox software.

3 Results

3.1 Extraction process optimization

From a range analysis (Table 1), it was concluded that the ratio of material to liquid has the greatest influence on the total polysaccharide content of the extract, the influence of the extraction temperature is second greatest, and the influence of the extraction time is the smallest. In summary, the total polysaccharide content was taken as the parameter to be optimized. The optimum extraction ratio was 1:100, the extraction temperature was 100 °C, and the extraction time was 3 h.

Table 1
Orthogonal test results of plant moisturizing composition extraction

No.	Factor			Total polysaccharide content[#] (mg/g)
	Material-to-liquid ratio (A)	Temperature/°C (B)	Extraction time/h (C)	
1	1	1	1	30.93
2	1	2	2	26.68
3	1	3	3	31.60
4	2	2	3	83.07
5	2	3	1	101.68
6	2	1	2	47.49
7	3	3	2	149.66
8	3	1	3	118.17
9	3	2	1	112.65
K1	29.74	65.53	81.75	
K2	77.41	74.13	74.61	
K3	126.82	94.31	77.61	
Range	97.08	28.78	7.14	

3.2 Results Of The Stability Study

Continuously and regularly detecting changes in the polysaccharide content of the same batch of plant moisturizing composition over time showed that the polysaccharide content was stable.

The results show that under the above various conditions, the plant moisturizing composition had good stability in the stock solution, no obvious color change occurred within 24 months, the technical indexes were stable and no precipitation occurred.

The plant moisturizing composition had good stability in creams, gels, and essences (final concentration 5%), and no instability, such as discoloration or stratification, was present.

3.3 Results Of The Keratinocyte MTT Assay

The MTT assay results (Table 2) indicate that the maximum safe concentration of the plant moisturizing composition for keratinocytes was 500 ppm.

Table 2
MTT test results for plant moisturizing composition

Cell viability	Concentration (ppm)									
	15.6	31.3	62.5	125	250	500	1000	2000	4% DMSO	/
Average value%	99.20	98.28	96.81	97.73	92.88	90.55	71.47	62.70	21.78	100.00
Standard variance%	5.60	3.97	4.59	3.59	4.04	5.99	5.78	0.84	4.62	2.77

3.4 FLG And CLDN-1 Cell Immunofluorescence

The plant moisturizing composition was set at three concentrations (125 ppm, 250 ppm, 500 ppm) based on a dose of 500 ppm, and fluorescence quantitative PCR was carried out. The variation trend of the sample at different concentrations is shown in Figs. <link rid="fig2">2</link>–7 and 2–8. Figure 1 and Fig. 2 show that at the genetic level, the plant moisturizing composition had a significant effect on the expression of FLG and CLDN-1 at doses of 250 ppm and 500 ppm.

3.5 AQP3 Cell Immunofluorescence

The results showed that the average fluorescence intensity of AQP3 protein expressed by keratinocytes after plant moisturizing composition treatment was significantly higher than that of the blank control group[18]. An immunofluorescence image of the AQP3 protein is shown in Fig. 3. The results showed that at the protein level, the plant moisturizing composition significantly improved the expression of AQP3 at a dose of 500 ppm.

3.6 Results Of The Erythrocyte Hemolysis Test

Table 3
Red blood cell hemolysis rate of plant moisturizing composition

Sample	Erythrocyte hemolysis rate (%)	Post-centrifugation
1% plant Moisturizing composition	1.2	Upper layer is light yellow and transparent, bottom has sedimentation
5% plant Moisturizing composition	1.5	Upper layer is light yellow and transparent, bottom has sedimentation
10% plant Moisturizing composition	1.8	Upper layer is light yellow and transparent, bottom has sedimentation
0.02%SDS	100	Uniform transparent red, a small amount of precipitate at the bottom

The erythrocyte hemolysis test results prove that the plant moisturizing composition has a very low red blood cell hemolysis rate at concentrations below 10% and no stimulating effect. (Table 3)

3.7 Results Of The 3T3 Neutral Red Test

The judgment criteria are shown in Table 4. The PC OD540 values were all larger than 0.4, and the PC CPZ hydrochloride met the criteria (IC50 (+ UV) was in the range of 0.1-3.0 μg/mL, the IC50 (-UV) was in the range of 7.0–90.0 μg/ml, and PIF ≥ 6) to indicate that the experiment was established. The MPE was obtained by comparing the cytotoxic concentration response curve obtained with light (+ UV) and without light (-UV) on the concentration grid; the concentration range was in the light (+ UV)) and a range of concentrations common in the no-light (-UV) test. The MPE was obtained using the special software "PHOTO32".

Table 4
Determination criteria for in vitro 3T3 neutral red uptake phototoxicity

Experimental data	Result determination
PIF<2.0 or MPE<0.1	No expected phototoxicity
PIF>2.0 and <5.0 or MPE>0.1 and <0.15	No expected phototoxicity
PIF>5.0 or MPE>0.15	No expected phototoxicity
Note: PIF = IC50 (-UV)/IC50 (+ UV)	

The results of the 3T3 neutral red uptake phototoxicity test for the test plant moisturizing combination are shown in Table 5.

Under the conditions of this test, the PIF value of the plant moisturizing composition sample was *1, indicating that the test sample did not exhibit cytotoxicity at the highest allowable concentration value (1000.0 μg/mL). The MPE value of the sample was 0.04, i.e., less than 0.1, further indicating that the sample is expected to be phototoxic. Therefore, under the conditions of this experiment, the 3T3 neutral red uptake phototoxicity test without the plant moisturizing combination was negative, and no phototoxicity was expected.

Table 5
Results of the 3T3 neutral red uptake phototoxicity test outside the plant moisturizing combination

Test article/reference product	$IC_{50}/(\mu g \cdot mL^{-1})$		PIF	MPE	Phototoxicity
	no light (-UV)	light (+ UV)			
Positive control CPZ	16.51	0.29	22.34	0.41	+
Plant moisturizing composition	> 1000.0	> 1000.0	*1	-0.04	-
Note: *1: No cytotoxicity was achieved when the highest allowable concentration value (1000.0 μg/mL) was reached; +: phototoxicity is expected; -: no phototoxicity is expected.					

4 Discussion

The plant moisturizing composition was optimized through a small number of experimental procedures. The optimal conditions were as follows: the material-liquid ratio was 1:100, the extraction temperature was 100 °C, and the extraction time was 3 h. According to the production process for this small sample, the scale of a pilot test was enlarged, and trial production proved that the process is feasible and the quality of the produced product meets its requirements, which is basically consistent with the indicators from the small sample.

By studying the effect of plant moisturizing composition on the expression of FLG, CLDN-1 and AQP3, the effect on skin moisture content and the improvement of overall skin moisturization, it was confirmed that the plant moisturizing composition developed by this project has the ability to repair the stratum corneum barrier and promote moisturization. The production and transportation of water makes the skin plump, delicate and moisturized.

The red blood cell hemolysis test and 3T3 phototoxicity test results show that the plant moisturizing composition is safe and causes no irritation to the skin, no phototoxicity, and no adverse reactions. The plant moisturizing composition can be used as a moisturizing raw material in cosmetics.

5 Conclusion

Therefore, to solve the problem of dry skin, we must pay attention to repairing the skin barrier and reducing skin inflammation while replenishing the moisture content of the skin. In addition, it is necessary to increase the amount of skin oil and fat and adjust the skin water and oil balance. From the perspective of

modern mechanisms, the plant moisturizing composition mainly solves the problem of skin dryness by maintaining skin barrier function, increasing endogenous water production and promoting endogenous water transport. From the perspective of traditional Chinese medicine, there are four ways to solve the problem of dry skin: “solid water screen", “rehydration”, “heat-clearing and anti-inflammatory", and "nurturing and nourishing yin" activities.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FanYI, Hong MENG and Jia-Rui LI have drafted the work; Yin-Mao Dong and Hong Meng designed the work; Fan Yi, Zhao-Hui QU and Han-Kun REN interpreted of data; You-Ting LIU, Yi-Fan HE, Wei-Hong ZHANG and Li LI substantively revised it. All authors read and approved the final manuscript.

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Figures

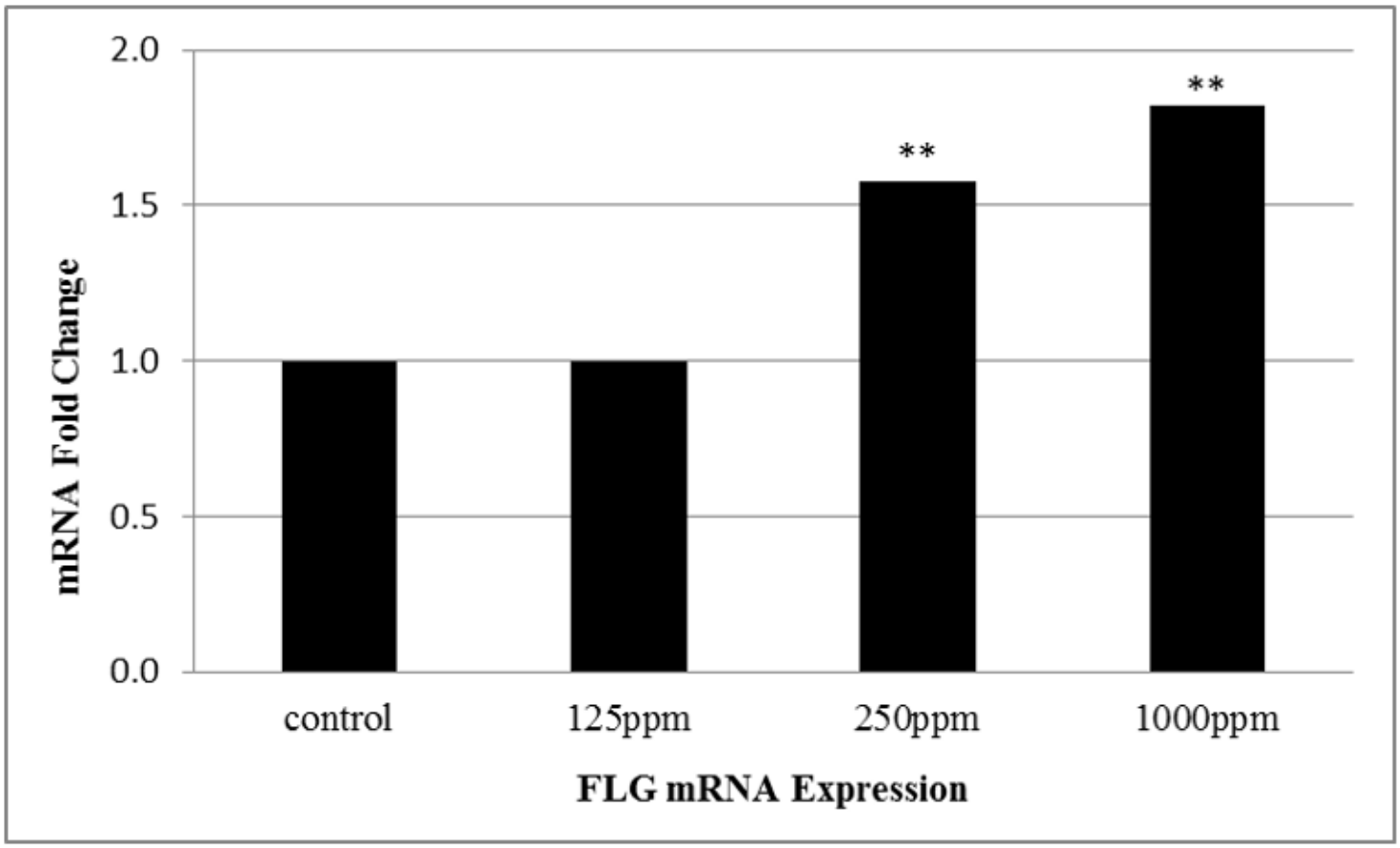


Figure 1

Effect of plant moisture composition on FLG expression

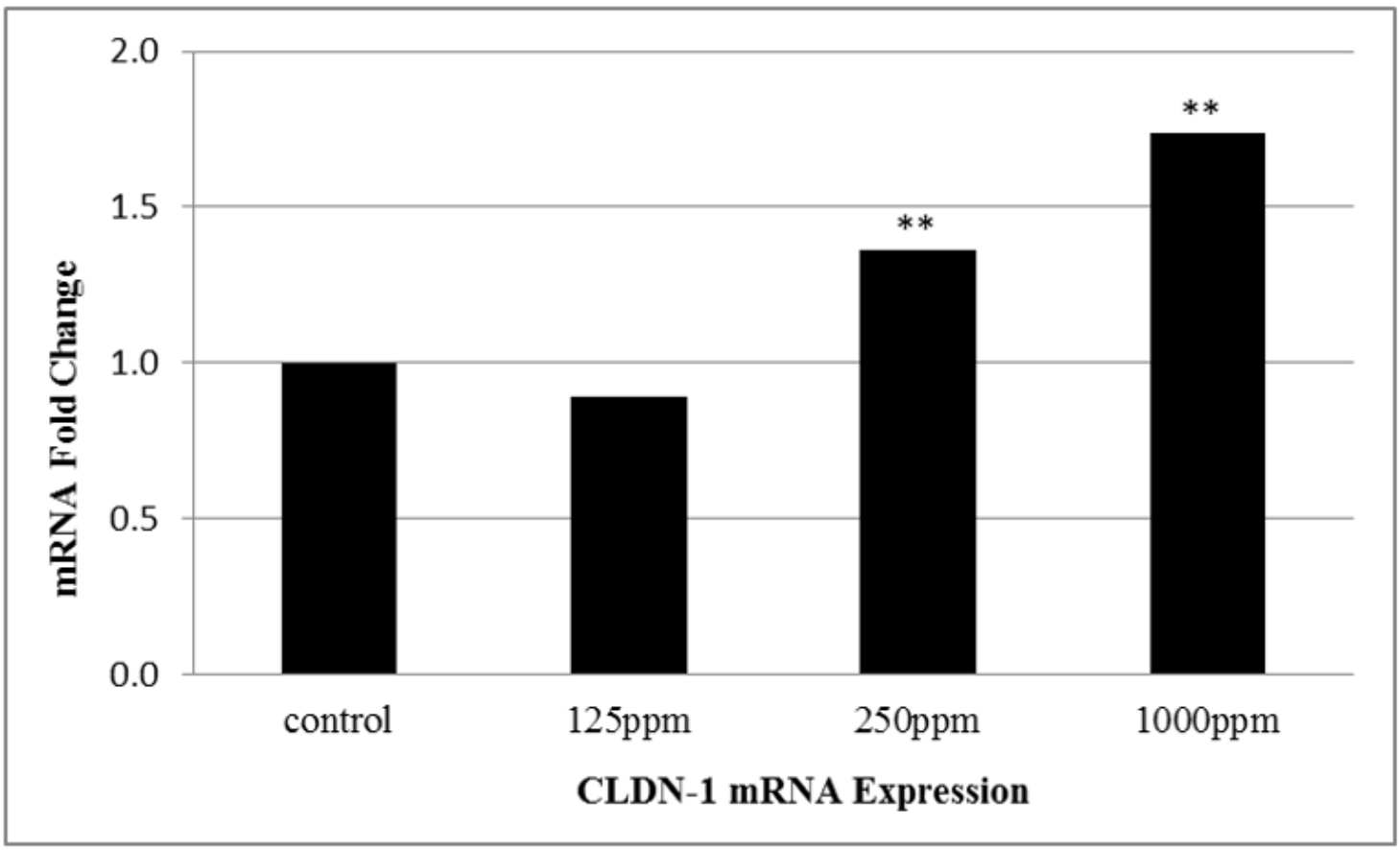
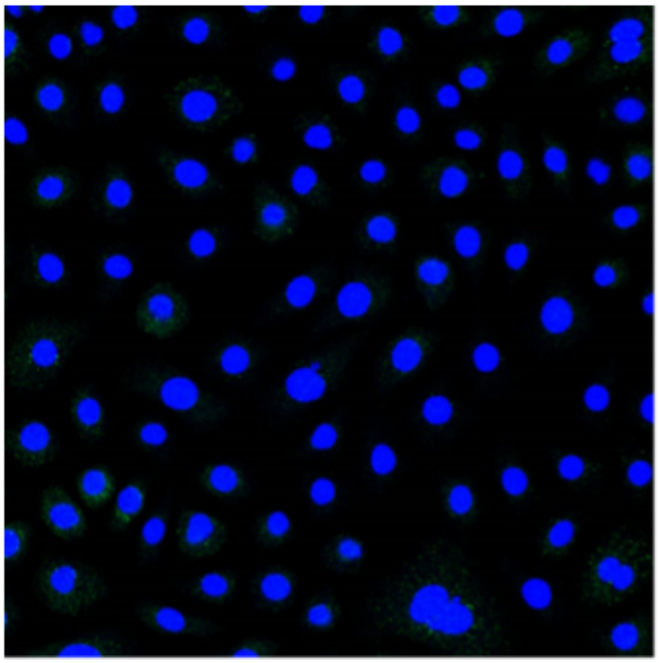


Figure 2

Effect of plant moisture composition on the expression of CLDN-1 Note: Using SPSS Dunnett's t test analysis, * and ** indicate that the plant moisturizing composition values were significantly different ($p<0.05$) and extremely significantly different ($p<0.01$) from the control group values.



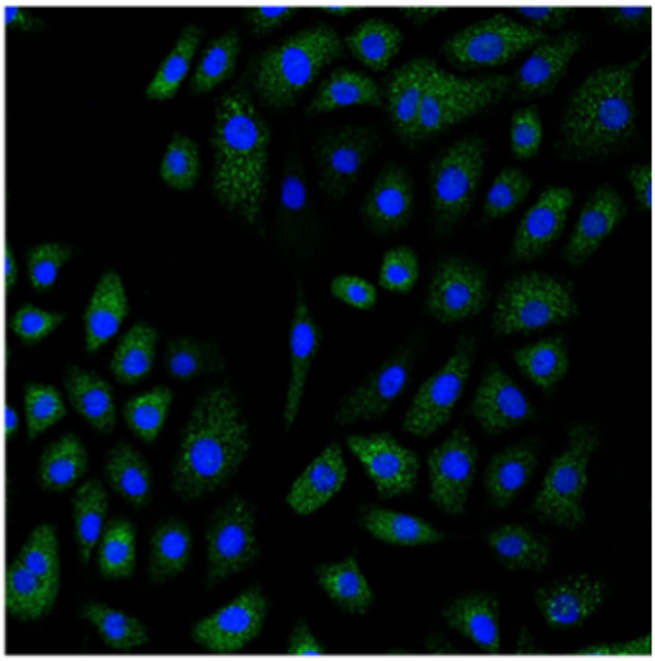


Figure 3

Sample AQP3 immunofluorescence photo Note: The green fluorescence indicates AQP3 protein expression, and the blue fluorescence indicates Hoechst dye staining sites (nuclear regions). The left picture shows the control group, and the right picture shows the experimental group.