IFSCC 2025 full paper (ABSTRACT N° IFSCC2025-1072)

Study of lophatherum gracile extract in relieving skin sensitivity

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1.Introduction.

Skin sensitivity refers to a highly reactive state of the skin under physiological or pathological conditions, where the skin is prone to exhibit signs of redness, swelling, itching, heat, pain, etc., along with a series of reactions such as oxidative stress and increased inflammatory factors in response to stimuli from UV radiation, physical and chemical substances, and other external factors affecting the skin [1]. In recent years,reports on skin sensitivity have become increasingly prevalent and concerning [2], especially in recent years due to the widespread use of face masks as a preventive measure against COVID-19. The direct contact and friction between facial skin and masks have exacerbated damage to the skin barrier leading to an increase in instances of sensitive skin.

TRPV1, a non-selective cation channel permeable to calcium ions plays a crucial role in diagnosing and treating sensitive skin. It can be specifically activated by capsaicin, the principal active component found in peppers, hence it is also known as the capsaicin receptor [3]. TRPV1 receptors are present not only at nociceptive sensory nerve endings but also within other cells of the epidermis such as keratinocytes and mast cells [4-7], being responsive to various physical, chemical or thermal stimuli [8]. Therefore it becomes imperative for raw materials or cosmetics designed for sensitive skins to target TRPV1 activation.

Lophatherum gracile, a traditional Chinese medicinal herb, is obtained from the dried stems and leaves of *Lophatherum gracile* Brongn., a plant belonging to the Gramineae family. It has a sweet and mild flavor, exhibits cold properties, and is renowned for its ability to clear heat, alleviate restlessness, promote diuresis, and dispel fire [9]. In this study special extraction techniques were employed for preparing *Lophatherum gracile* extract which was then evaluated both in vitro studies as well as human trials regarding its efficacy and safety. This research aims at supporting further development & application of *Lophatherum gracile* extract within cosmetic formulations.

2.Materials and Methods.

Materials

Lophatherum gracile (Huangshan, Anhui, China) whose officinal parts are leaves/stems.

Absolute ethyl alcohol , sodium carbonate , gallic acid (chromatographically pure, purity ≥ 98%), foline-phenol reagent , highly TRPV1-expressing HaCaT cells (HaCaT-TRPV1-OE cells); capsaicin; capsazepin; sodium dodecyl sulfate (SDS); DMEM high-glucose medium, penicillin-streptomycin double antibody solution, trypsin; CCK-8 Kit; mouse monocyte chemoattractant protein-1 (MCP-1) Elisa kit and human interleukin-8 (IL-8) Elisa kit; SPF white leghorn egg. Inverted microscope; microplate reader; cell counter, CO_2 incubator, biosafety cabinet; horizontal oscillator; TM-300 skin moisture loss test probe, Tewameter TM HEX transepidermal water loss (TEWL) tester, and Colorimeter CL400 skin color test probe; Antera 3D; ultraviolet spectrophotometer, Angra EU-2600D.

Preparation of *Lophatherum gracile* extract

An appropriate amount (100 g) of *Lophatherum gracile* stems and leaves was taken, crushed to 80 meshes, and then mixed with deionized water at a material-liquid ratio of 1:20. The mixture was heated at 90°C for 2 hours, filtered through a 5 μm polypropylene membrane to collect the filtrate as the crude *Lophatherum gracile* extract. The crude Lophatherum gracile extract was then combined with a 30% ethanol solution at a material-liquid ratio of 1:20. The resulting mixture was loaded onto a polyamide chromatographic column for adsorption, followed by gradient elution using deionized water, 40% ethanol solution, and finally an 80% ethanol solution. The eluates were collected and pooled, concentrated until no alcohol smell remained, mixed with butanediol, and filtered to obtain the final *Lophatherum gracile* extract.

Cytokine testing

The HaCaT-TRPV1-OE cells were cultured in complete DMEM medium at 37°C and 5% CO2. After harvesting, washing, and trypsin treatment, the cells were re-suspended and inoculated into a 96-well plate for incubation. The test substance was diluted to prepare 6 concentrations for later use. Capsaicin and capsaicin were used as stimuli and positive controls respectively. After adding the test substance and stimulator (Table 1), the supernatant was collected after 24 hours of culture for cytokine detection using ELISA kit. The formula for calculation of the cytokine inhibitory rate is as follows:

Inhibition rate = (expression $level_{model\ group}$ - expression $level_{test\ group}$)/expression $level_{model\ group}$ × 100%

Table 1 Test method of HaCaT-TRPV1-OE cell.

Group	Sample	Concentration	Stimulator	Index
Blank control	/	/	/	IL-8、MCP-1
Model group	Complete culture medium	/	Capsaicin（2×10^{-5} mol/L）	
Positive group	Capsazepin	1×10^{-5} mol/L		
Sample group	*Lophatherum gracile extract*	0.003%		
		0.01%		
		0.03%		
		0.1%		

	0.3%	
	1.0%	

Formula and preparation process

Referred to Table 2 for the mass fractions of each component added in the emulsion formula containing *Lophatherum gracile* extract.

Preparation process: The A-phase raw materials were heated to 70–80°C and stirred until fully dissolved; the B-phase raw materials were dispersed at room temperature, gradually added into the A-phase, homogenized at 70-80°C for 10 min under high pressure, then cooled to room temperature; subsequently, the C-phase raw materials were added and stirred until completely dissolved prior to filling.

Table 2 Formula ingredient table.

Phase	Ingredient（INCI）	1#	2#	3#	4#	5#
A	WATER			46		
	BUTYLENE GLYCOL			6		
	DISODIUM EDTA			0.05		
	CELLULOSE GUM			0.2		
	HYDROXYACETOPHENONE			0.3		
	PENTYLENE GLYCOL			0.5		
B	PPG-4-CETEARETH-20			1		
	CETEARETH-21			2.5		
	CETEARYL ALCOHOL			3.5		
	GLYCERYL STEARATE			0.5		
	ETHYLHEXYL PALMITATE			4		
	CYCLOPENTASILOXANE			6		
C	*LOPHATHERUM GRACILE* EXTRACT	/	0.5	1	2	5
	WATER			Up to 100		

SDS stimulation-based skin injury model test

The tests were conducted in accordance with the Declaration of Helsinki. All test samples passed the skin safety test, and all recruited subjects signed informed consent forms reviewed by the Ethics Committee of Cosmax (Shanghai) Testing Technology Co., Ltd.

Test method for efficacy measurement of skin soothing cosmetic products" (T/GDCDC 021-2022) was used. Subjects meeting the inclusion criteria cleansed their faces and rested in a controlled environment with constant temperature and humidity for 30 minutes. Five random test sites were marked on each subject's forearm, and initial values of transepidermal water loss (TEWL) and erythema index (EI) were measured as blank controls. Test substances 1-5#

were applied to the test sites, with Formula 1# serving as a negative control and Formulas 2-5# serving as sample groups. Patch tester chambers filled with inducer were then applied to the subjects' test sites using hypoallergenic tapes, left in place for 24 hours before removal.
After the period of inducing skin erythema reaction, the condition of the induced skin at the test site was assessed and examined by professionals. The test sites with poor consistency were excluded, and the TEWL value and EI were measured. A higher TEWL value indicates greater water loss through the epidermis, while a higher EI value indicates more pronounced skin redness.

Lactic acid stimulation-based skin injury model test

Subjects from the internal subject database who self-rated as having sensitive skin according to the questionnaire and were found to be sensitive to lactic acid were selected (with a minimum of 30 effective cases). Professionals randomly applied 50 μL of 10% lactic acid solution on either side of the cheek's nasolabial groove. The degree of tingling was scored at 0, 2.5, and 5.0 minutes after application using a 4-point method (0: no tingling, 1: mild tingling, 2: moderate tingling, and 3: severe tingling). A higher score indicated a stronger sensation.
After following the test procedure and resting in a controlled environment for 30 minutes (21±1°C, 50%±10%), baseline values for TEWL and stratum corneum water content were measured. A higher TEWL value indicates greater water loss through the epidermis, while higher water content in the stratum corneum suggests a more effective skin barrier. The subjects applied Formula 4# (2% *Lophatherum gracile* extract) to their entire face once in the morning and once in the evening for 28 days. The indicators were assessed after product use for both 14 and 28 days, with a repeat of the lactic acid stinging test on day 28.

Capsaicin stimulation-based skin injury model test

The subjects from the internal database were those who self-rated as having sensitive skin according to a questionnaire, with at least 30 effective cases. After following the test procedure and resting in an environment of constant temperature and humidity (21±1°C, 50%±10%) for 30 minutes, two test sites were marked on their forearms. Each site was then applied with 50 μL of 0.005% capsaicin solution to stimulate erythema, followed by application of 100 μL of Formula 1# (without Lophatherum gracile extract) and Formula 4# (with 2% Lophatherum gracile extract), respectively. At intervals of 0,5,10,and30 minutes after application, the subjects rated discomfort at each test site using a scale ranging from "0" for no discomfort to "5" for painful sensation. Erythema images were obtained and analyzed using Antera 3D.

Data analysis

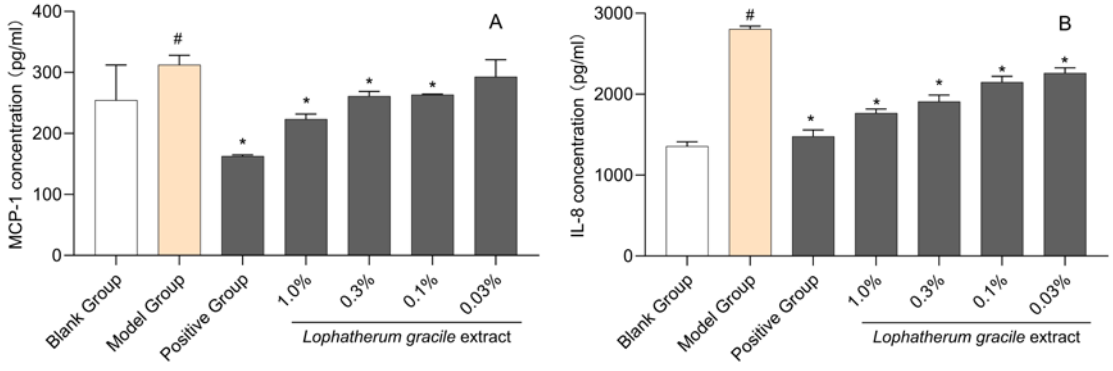
SPSS software was used for statistical analysis. T-test or nonparametric test was performed for statistical analysis according to whether the test data conformed to normal distribution, with the test level of 0.05, and $p<0.05$ was considered statistically significant.

3.Results.

Cytokine testing

As shown in Figures 1 (a) and 1 (b), the expression levels of inflammatory factors MCP-1 and IL-8 were significantly upregulated ($^{\#}p<0.05$) following capsaicin stimulation in HaCaT-TRPV1-OE cells, indicating successful establishment of the model. Subsequent treatment with varying

concentrations of *Lophatherum gracile* extract resulted in a dose-dependent reduction in the expression levels of MCP-1 and IL-8(*p<0.05). These results demonstrate that 0.1%-1.0% *Lophatherum gracile* extract effectively inhibits capsaicin-induced increase in MCP-1 and IL-8 in TRPV1 overexpression cells.



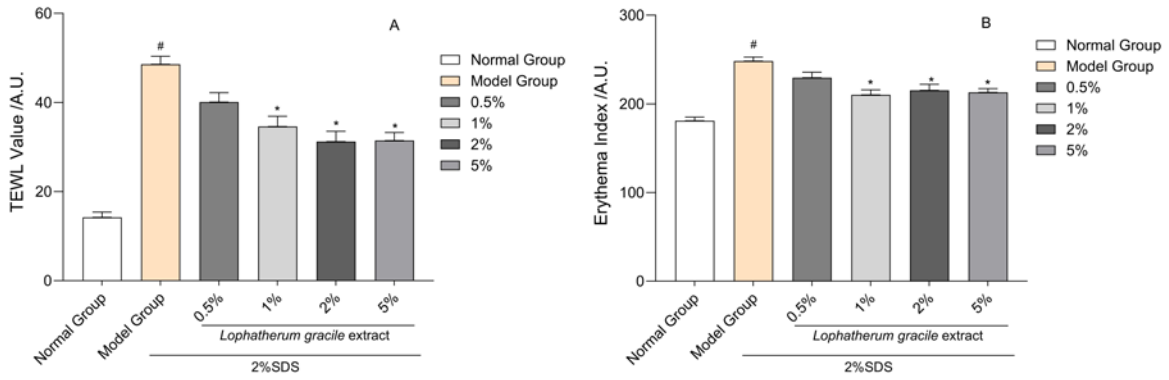
(a) **(b)**

Figure 1 Effects of *Lophatherum gracile* extract on cytokines.(a)Effects of *Lophatherum gracile* extract on MCP-1.(b)Effects of *Lophatherum gracile* extract on IL-8.（#p<0.05 compared with blank group; *p<0.05 compared with model group）

SDS stimulation-based skin injury model test

Physical or chemical stimuli can cause damage to the human skin barrier and establish a human skin model for evaluating soothing effects [10]. Sodium dodecyl sulfate (SDS) is a widely used surfactant in cosmetics, which can lead to abnormalities in cell membrane proteins. SDS with a mass fraction above 0.5% can result in skin discomfort. In this study, a skin injury model was created using a 2% SDS solution as a stimulus, and the Lophatherum gracile extract's soothing effect on injured skin was evaluated based on TEWL value and EI value.

Figures 2(a) and 2(b) show that applying 2% SDS significantly increased TEWL value by 241.73% and EI value by 37.09% compared to the normal group, indicating successful model establishment. Treatment with *Lophatherum gracile* extract reduced TEWL and EI values compared to the model group (Formula 1# without *Lophatherum gracile* extract). Specifically, TEWL values for Formula 3#, Formula 4#, and Formula 5# decreased by 28.73%, 35.71% and 35.21%, respectively, while the corresponding EI values decreased by 15.36%, 13.30% and 14.16%. These findings suggest that *Lophatherum gracile* extract with a mass fraction exceeding 1% effectively enhances skin barrier function.



(a) **(b)**

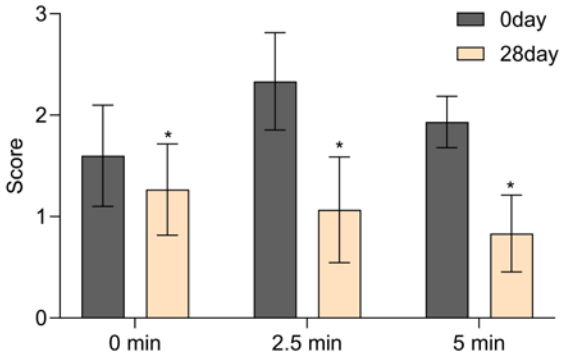
Figure 2 Results of damaged skin after product application.(a)TEWL value results of damaged skin after product application.(b)EI value results of damaged skin after product application. (#p<0.05 compared with normal group; *p<0.05 compared with model group)

Lactic acid stimulation-based skin injury model test

The lactic acid stinging test is a semi-subjective method for evaluating sensitive skin, in which subjects may experience tingling and other discomforts during the test. In this study, a 10% lactic acid solution was used to induce sensitivity in the skin, and the soothing and repairing effects of *Lophatherum gracile extract* on sensitive skin were assessed using indicators such as lactic acid stinging score, TEWL value, and stratum corneum water content. Based on preliminary tests and Ethics Committee discussions, Formula 4# (containing 2% *Lophatherum* gracile extract) was chosen for conducting the lactic acid-stimulating skin injury test.

As shown in Figure 3, the scores following lactic acid stimulation decreased significantly by 20.83%, 54.29% and 46.02% at 0, 2.5 and 5 minutes, respectively after the application of Formula 4# on day 28, all of which were statistically significant (*p<0.05). This indicates that the extract of *Lophatherum gracile* could promptly alleviate and soothe skin irritation caused by lactic acid.

According to the data presented in Table 3, *Lophatherum gracile* extract has the potential to enhance the barrier function of sensitive skin.



Figures 3 Lactic acid stinging score of skin after product application.
(*p<0.05 compared with 0 day)

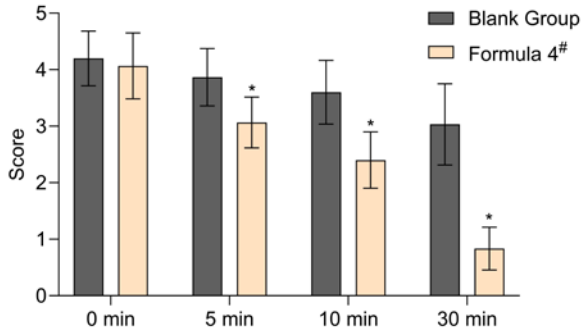
Table 3 Tewl value and water content results of skin after product application.

	0 Day	14 Day	28 Day
TEWL value（g/cm^2）	25.02±3.40	23.13±2.95	22.06±2.84
Water content（A.U.）	49.51±5.58	55.34±5.38	58.24±6.58

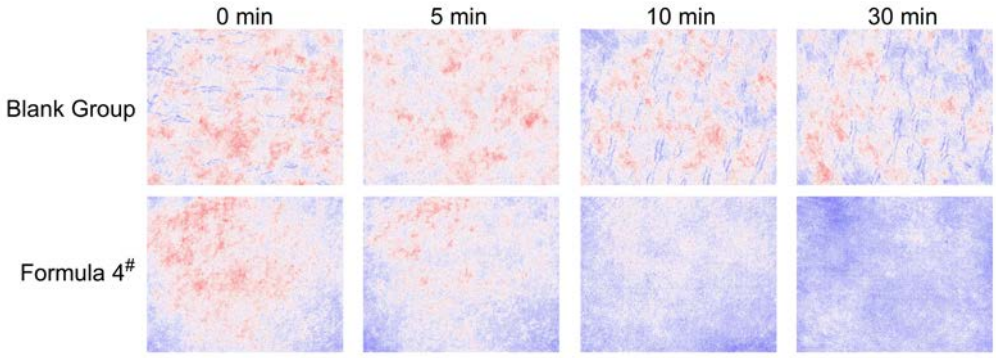
Capsaicin stimulation-based skin injury model test

The binding of capsaicin to the capsaicin receptor (TRPV1) leads to the influx of calcium and sodium ions, as well as the synthesis and release of various neurotransmitters such as substance P and calcitonin gene-related peptides (CGRPs), inducing neurogenic inflammation that causes skin discomforts like burning, itching, tingling, and swelling. Therefore, it is commonly used for assessing sensitive skin [11]. In this study, a 0.005% mass fraction of capsaicin was used to establish a sensitive skin model stimulated by capsaicin. The effects of *Lophatherum gracile* extract on sensitive skin were evaluated based on discomfort scores and skin erythema pictures of the subjects. Following preliminary tests and discussion with the Ethics Committee, Formula 4$^{\#}$ was selected for conducting the capsaicin-stimulating skin injury test.

As depicted in Figure 4, the discomfort reaction at the site treated with Formula 4$^{\#}$ were significantly alleviated, with a reduction in discomfort score by 24.59%, 40.98% and 79.51% at 5, 10 and 30 minutes post-application compared to the blank group, all of which were statistically significant (*p<0.05). As depicted in Figure 5, the erythema reaction at each time point on the site treated with Formula 4$^{\#}$ was markedly lower than that on the blank group site, indicating that *Lophatherum gracile* extract could rapidly alleviate skin discomfort and redness symptoms induced by capsaicin in subjects, suggesting its potential for preventing and promptly addressing sensitive skin issues.



Figures 4 Discomfort scores of skin after product application
(*p<0.05 compared with blank group)



Figures 5 Erythema photograph after product application

4.Discussion.

Sensitive skin is widespread in the world, with Chinese women accounting for over 36.1% of this group. It is projected that by 2030, this figure will increase to 48%. Currently, the mechanism underlying sensitive skin remains incompletely understood but is primarily associated with impaired skin barrier function alongside inflammatory responses and hyperreactive neurovascular systems [12] .

Numerous studies [13-15] have demonstrated that TRPV1 can serve as a diagnostic marker for sensitive skin and is also a key target in the induction of muscle sensitivity. Zhou Lidan *et al* [16] discovered that capsaicin can activate TRPV1 channel, leading to increased expression of cytotoxic cytokines IL-8 and MCP-1 in a comparative study of highly TRPV1 expressing cells (HaCaT-TRPV1-OE) and normal HaCaT cells. In this study, HaCaT-TRPV1-OE was utilized as an in-vitro substitute model for sensitive skin to investigate the characteristics of the *Lophatherum gracile* extract.

The skin barrier injury and discomfort symptoms were artificially induced using chemical reagents such as SDS, lactic acid, capsaicin, and other stimuli to simulate the sensitive skin state. Subsequently, the efficacy of *Lophatherum gracile* extract in improving sensitive skin was assessed from both subjective and objective clinical perspectives.

5.Conclusion.

The effect of *Lophatherum gracile* extract on sensitive skin was assessed in vitro and in human clinical studies. Using HaCaT cells with high TRPV1 expression as a cell model, it was observed that the Lophatherum gracile extract significantly inhibits TRPV1 activation, reduces MCP-1 and IL-8 inflammatory factor expression, and alleviates inflammatory reactions caused by external stimuli. Skin barrier injury and discomfort symptoms were artificially induced using chemical reagents such as SDS, lactic acid, capsaicin, to simulate the sensitive skin state.

In this study, the impact of *Lophatherum gracile* extract on sensitive skin was evaluated through in vitro and human clinical studies. Employing HaCaT cells as a cell model with heightened TRPV1 expression, it was observed that the *Lophatherum gracile* extract significantly suppresses TRPV1 activation, diminishes MCP-1 and IL-8 inflammatory factor expression, and mitigates inflammatory responses induced by external stimuli. Skin barrier impairment and discomfort symptoms were artificially induced using chemical reagents such

as SDS, lactic acid, capsaicin to replicate the state of sensitive skin. The findings have demonstrated that the *Lophatherum gracile* extract can expedite the healing process of damaged skin and promptly alleviate skin discomfort such as tingling and redness; with prolonged use, it can decrease the TEWL value of the skin and enhance its moisture content. In summary, the *Lophatherum gracile* extract is capable of effectively mitigating the sensitivity of sensitive skin caused by external stimuli and facilitating the restoration of the skin barrier.

Acknowledgments.
Authors would like to acknowledge the support of Cosmax (Shanghai) Testing Technology Co., Ltd. in conducting clinical trials.

Conflict of Interest Statement.
All subjects had given their informed consent to participate.

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