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"TARGETING NEUROGENIC INFLAMMATION AS A SOLUTION FOR HYPERSENSITIVE SKIN"

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1. Introduction

The inflammatory response of the skin is of a great interest in the cosmetics industry. Inflammation is the immune system's response to a damage caused to cells or tissues by bacterial pathogens or by any other biological, chemical, physical, or mechanical aggressor. The classic symptoms of inflammation are pain, heat, redness, swelling and loss of function. Inflammation is a generic response and therefore it is considered a mechanism of innate immunity, compared to adaptative immunity, which is specific for each pathogen [1]. The first step of inflammation is known as irritation. It is a state of inflammation or painful reaction to allergy or cellular damage. The stimulus inducing this state can be chemical (examples: phenol and capsaicin), mechanical, thermic, or radioactive.

Cutaneous neurogenic inflammation (CNI) refers to inflammation in the skin that is triggered or exacerbated by the excessive release of neuropeptides from sensory nerve endings. This process leads to clinical symptoms primarily involving sensory and vascular disturbances, such as pruritus (itching) and erythema (redness) [2].

Cutaneous neurogenic inflammation (CNI) can be initiated by two main mechanisms: direct activation of sensory nerve endings by environmental stimuli, and indirect activation via upstream inflammatory or stress-related pathways [3–5]. Central to both mechanisms is the Transient Receptor Potential Ankyrin 1 (TRPA1) channel, a receptor expressed on sensory neurons that mediates pain, itch, and inflammation. TRPA1 is activated by cold, chemical irritants (e.g., allergens, capsaicin), and endogenous inflammatory mediators (e.g., cytokines) [6-7], leading to calcium influx and a cascade of intracellular signals that contribute to neurogenic inflammation and sensory discomfort.

Therefore, the activation of TRPA1 may occur through direct interaction with exogenous compounds with the channel itself or indirectly through inflammatory reaction, reactive oxygen species (ROS) generation, or skin barrier disruption, all of which enhance its expression or

sensitivity. In this way, TRPA1 plays a pivotal role in hypersensitive skin, a condition characterized by exaggerated responses to non-noxious stimuli, often in the absence of visible irritation.

Given its involvement in sensory perception and inflammation, TRPA1 has become a promising target for the treatment of skin disorders such as atopic dermatitis, psoriasis, and pruritus [8]. Modulating TRPA1 through both direct and/or indirect pathways offers promising opportunities for the development of innovative skincare treatments aimed at alleviating skin-related symptoms, enhancing skin comfort, and improving resilience against environmental stressors [9-10]. In this context, the development of cosmetic formulations containing active ingredients with anti-inflammatory, antioxidant, and barrier-supportive properties is of growing interest.

The present work aims to evaluate a novel cosmetic formulation containing *Ophiopogon japonicus* extract, cyanocobalamin (vitamin B12), and titrated *Centella asiatica* extract. These ingredients have demonstrated beneficial properties for sensitive skin, including anti-inflammatory activity, antioxidant protection, and enhancement of skin barrier integrity. We hypothesize that this combination may attenuate TRPA1 activation and thereby reduce neurogenic inflammation, contributing to greater skin comfort and resilience through the reinforcement of the epi-dermal barrier and mitigation of inflammatory and oxidative stress.

2. Materials and Methods

Formula development

Selection of raw materials included in this skincare composition and their content (%) were performed according to a reliable development process. The formulation is a O/W fluid emulsion containing *Ophiopogon japonicum* extract, cyanocobalamin and titrated *Centella asiatica* extract as active ingredients. Titrated Extract Centella Asiatica is a highly purified centella extract > 95% composed of powerful molècules genines asiàtic acid and madecassic acid, as well as triterpens asiaticoside.
The emulsion includes a renewable vegetable biomimetic emulsifier together with polyacrylate crosspolymer-6 and xanthan gum; it also contains a combination of natural origin emollients, silicones, and preservatives.

Ex vivo study to evaluate the effect of a formulation on TRPA1 expression after exposure to a stressor (capsaicin)

Human skin explants were subjected to a capsaicin treatment. A control condition that was not treated with capsaicin was included (NT). Control skin treated with capsaicin, but not treated with product was also included (C). The formulation was topically applied on human skin explants for 30 minutes and 1h. The protein expression level of TRPA1 was determined by immunostaining - confocal microscopy. After each incubation time skin explants were sectioned into pieces, introduced into scaffolds, cut using a cryostat and mounted on glass slides. Afterwards, sections were fixed and immunostained with anti TRPA1 antibody (Rabbit pAb) plus AlexaFluor 488-conjugated anti-rabbit, phalloidin Alexa fluor 633-conjugated (actin staining)

and DAPI (nuclei staining). Fluorescence images of each condition were obtained by confocal microscopy. Mean intensity fluorescence obtained from each of the pictures was normalized to the non-treated control. Data were statistically analysed.

Clinical evaluation

The study aimed to evaluate the efficacy in improving skin conditions related to sensitive skin. To achieve this goal, a study was conducted on 30 healthy Caucasian women and men between 20 and 65 years of age, with sensitive skin (with itching, redness and warmth sensation on face). They applied the product twice a day (morning and night) for 28 days. An informed consent was obtained by the volunteers to start the topical experimental study [11]. Assessments of the soothing efficacy on skin discomforts like erythema, itching and warm sensation were carried out at baseline (T0) and after 15 minutes by clinical evaluation following clinical score scale (**Table 1**):

Table 1. Clinical score scale

Discomfort evaluation at T0	Score	Soothing efficacy on skin discomfort at T15min	Score
No/None	**1**	No variation	**1**
Mild	**2**	Slight Improvement	**2**
Moderate	**3**	Moderate improvement	**3**
Severe	**4**	Remarkable improvement	**4**

Furthermore, the barrier function measurement was based in the measurement of the trans epidermal water loss (TEWL) by the recognized TEWAMETER ® method. The instrument used was a Tewameter 300® (Courage+Khazaka, electronic GmbH). Physical basis for the measurement was the Diffusion law discovered by Adolf Fick in 1855.

The instrumental and clinical analysis were further complemented with self-assessment questionnaires filled out by the subjects who completed the study.

Statistical analysis of instrumental measures was subjected to paired Student t-test (within-group analysis vs T0). Variations are considered statistically significant when the p value is <0.05.

3. Results

Formula development

The formulation process yielded a stable oil-in-water (O/W) emulsion with favorable physicochemical and organoleptic characteristics. The incorporation of the renewable vegetable biomimetic emulsifier allows the formation of liquid crystals that effectively maintained emulsion stability over time, ensuring homogeneity and preventing phase separation under standard storage conditions. The active ingredients—*Ophiopogon japonicum* extract, cyanocobalamin,

and *Centella asiatica* extract—were successfully solubilized and integrated into the external aqueous phase.

The final product exhibited a smooth, non-greasy texture, rapid absorption, and a pleasant sensorial profile, attributed to the combination of natural-origin emollients and emulsifiers and silicones. No significant changes were observed in pH, viscosity, or appearance during accelerated stability testing, indicating robust formulation performance.

These results confirm the feasibility of combining the selected active ingredients into a cosmetically elegant and stable vehicle, suitable for further evaluation in skin models targeting inflammation, sensitivity, and oxidative stress-related skin conditions.

Quantification of TRPA1 level by confocal microscopy

Results showed (**Table 2**), that the treatment with capsaicin induced the expression of TRPA1 by 54.9% ± 6.9% ($p<0.0001$). The formulation inhibits TRPA1 expression compared to capsaicin after 30 minutes and 60 minutes, with a reduction of 42.4% ± 6.7% ($p<0.0001$) and 99.1% ± 6.7% ($p<0.0001$), respectively (**Figure 1**).

Table 2: Results of TRPA1 levels after 30-and 60-min treatment with the formulation

Sample	Average	SD	VAR % vs C-
Non treated (NT)	0.4545	0.1185	-
Control (C)	1.024	0.1704	**+ 54.9%*** **
30 minutes	0.6655	0.2524	**- 42.4%*** **
60 minutes	0.009278	0.003731	**- 99.1%*** **

***for p-value < 0.0001

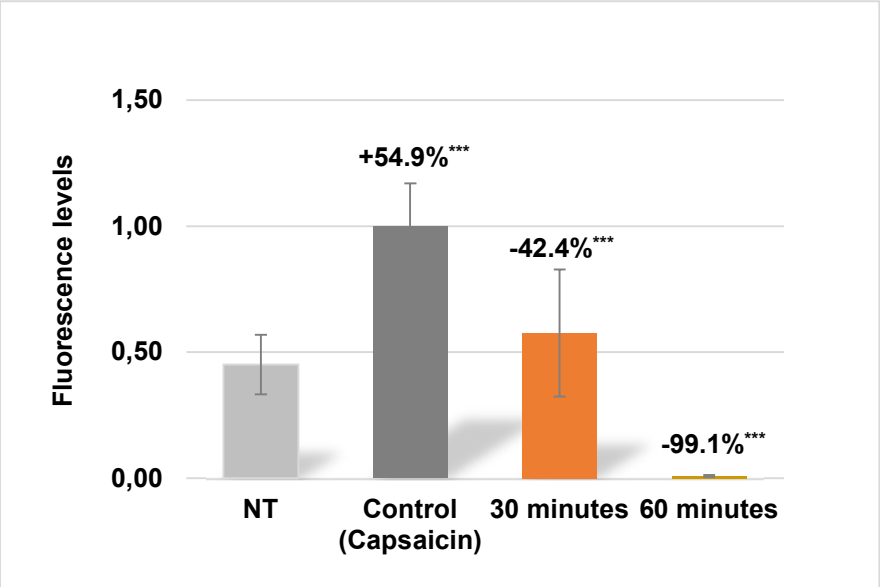

Figure 1. Graphical representation of the results showing the TRPA1 levels in the confocal microscope images after 30-and 60-min treatment with the formulation. Non treated control (NT), capsaicin only control (C). Asterisks represent statistical significance with **** for p-value < 0.0001).

A representative fluorescence images of each condition obtained by confocal microscospy are also shown in **Figure 2.** The protein expression level of TRPA1 (stained in green colour) demonstrate that at 1 hour the expression was almost reduced as no green colour was observed and therefore quantified by immunostaining.

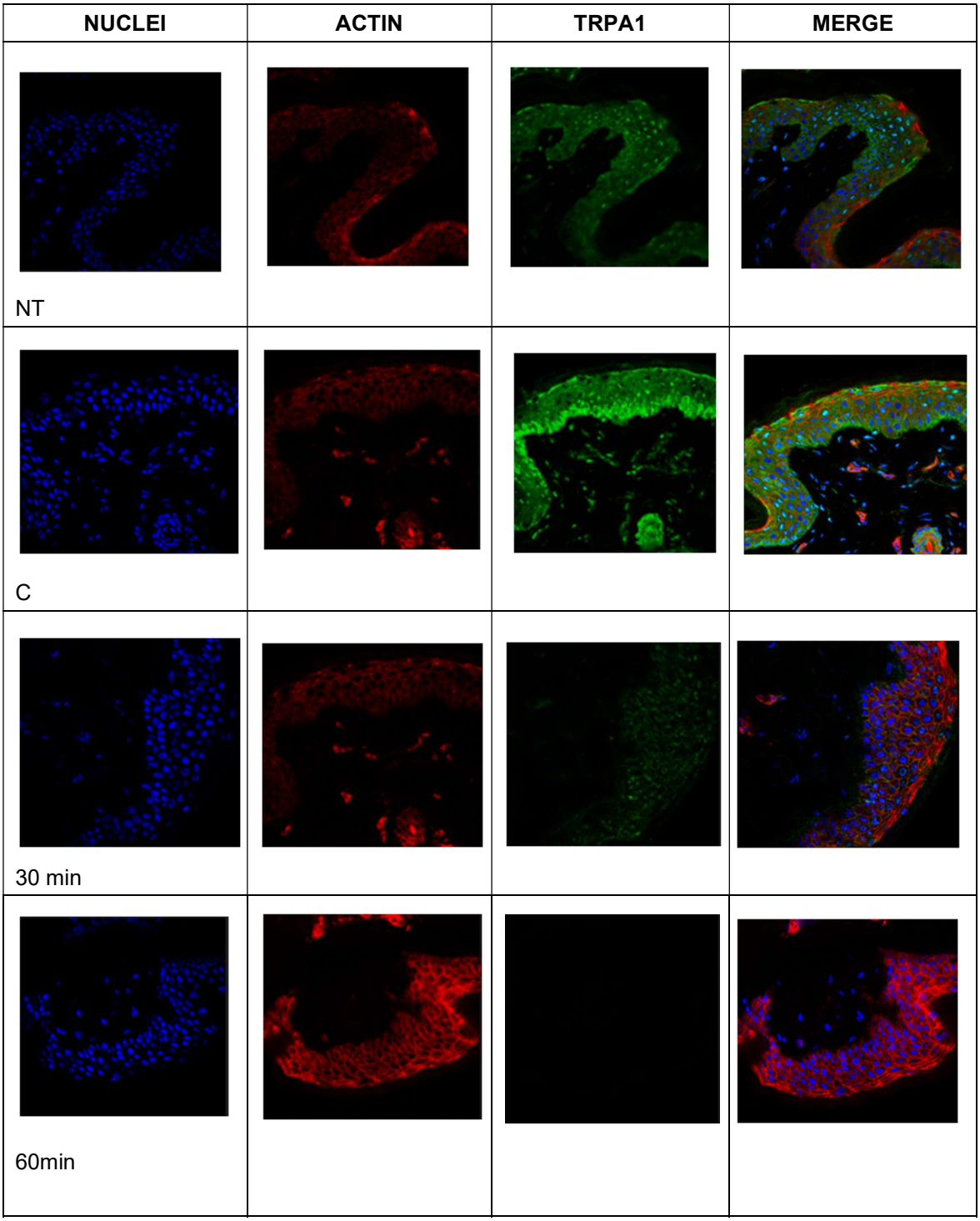

Figure 2. Representative confocal microscopy images of human skin explants after 30- and 60-min treatment with the formulation. Non treated control (NT), capsaicin only control (C). Nuclei are stained in blue, actin in red and TRPA1 in green.

Clinical evaluation

The efficacy of the formulation was evaluated on the volunteers clinically showing sensitive skin. The product was applied to their entire face, twice a day for 28 days.

The results in **Figure 3** show the transepidermal water loss (TEWL) before (T=0) and after (T=28) application of the formula. A significant decrease of the trans epidermal water loss parameter by an average of -10.0% can be seen. A decrease of TEWL parameter indicates an improvement of skin barrier function.

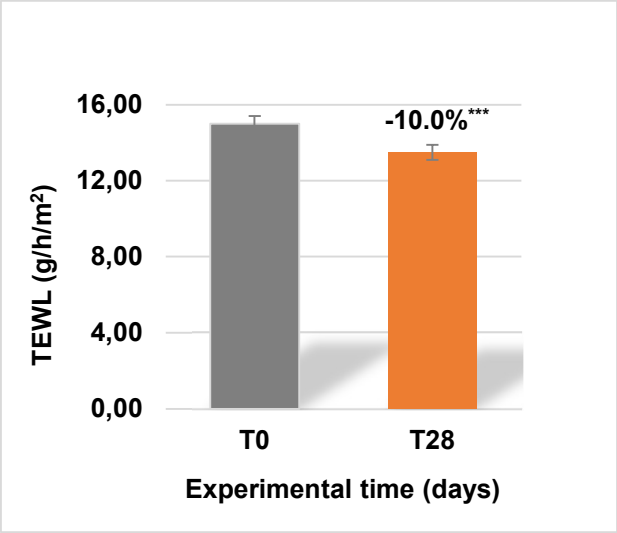

Figure 3: The graphic shows data obtained at each experimental time for the transepidermal water loss. Data are expressed as a mean ± SE. Above the error bar the inter-group statistical analysis vs. T0 is reported as follow: * p<0.05, ** p<0.01, *** p<0.001.

The results in **Figure 4** show the redness improvement before (T=0) and after (T=15min) application of the formula. A significant improvement of erythema (clinical evaluation) of +28.3% was observed.

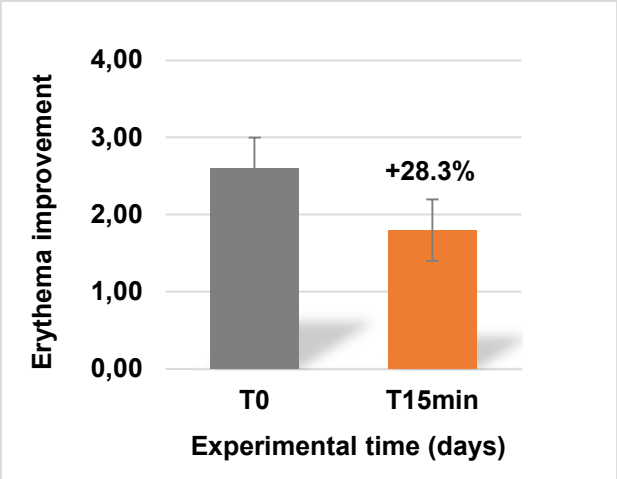

Figure 4: The graphics show the data obtained at each experimental time for erythema. Data are expressed as a mean ± SE.

Also, as shown in **Figure 5** shows a general improvement in terms of redness of a representative volunteer after applying the formula before (left) and after 28 days (right).

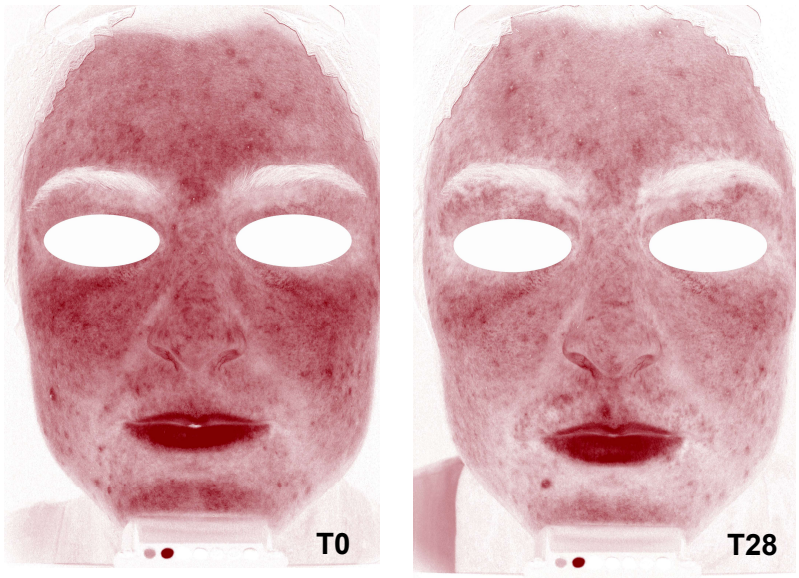

Figure 5: Images of 29 old volunteer who experienced an improvement in redness T0: pre-treatment evaluation; T28: post-treatment evaluation, after 28 days of formulation application.

The results in **Figure 6** show the warmth sensation improvement before (T=0) and after (T=15 min) application of the formula. A significant improvement in erythema (clinical evaluation) of +39.4% was observed.

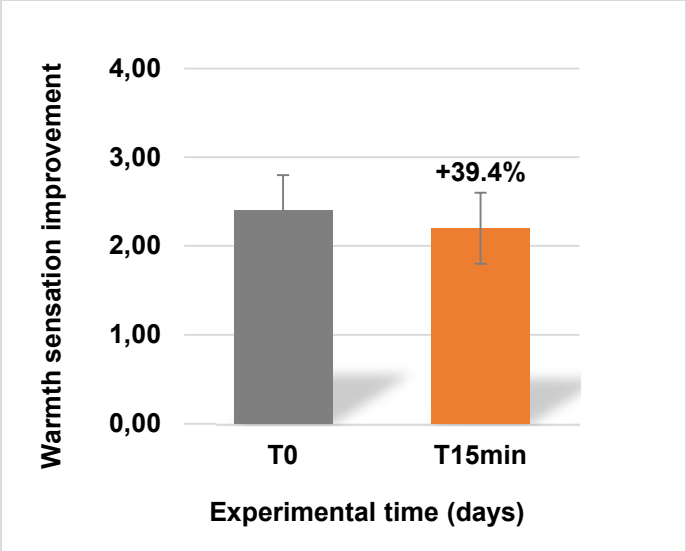

Figure 6: The graphics show the data obtained at each experimental time for warmth sensation. Data are expressed as a mean ± SE.

Finally, the results in **Figure 7** show the itching sensation improvement before (T=0) and after (T=15min) application of the formula. A significant improvement of itching (clinical evaluation) of +34.3% was observed.

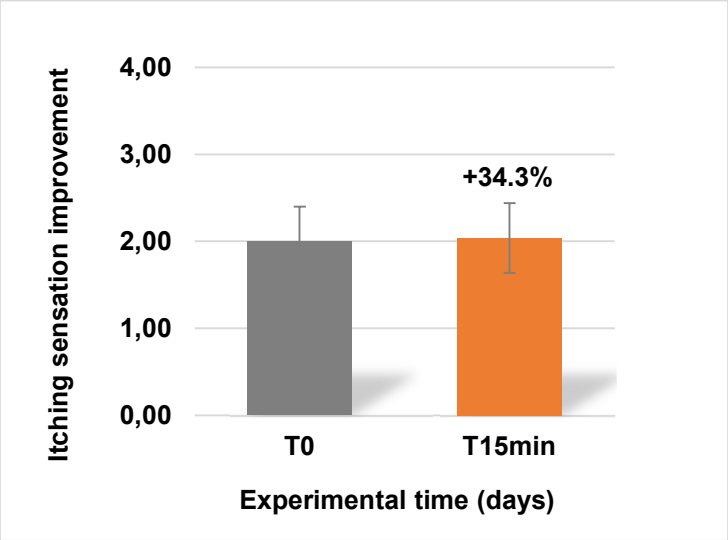

Figure 7: The graphics show the data obtained at each experimental time for itching sensation. Data are expressed as a mean ± SE.

4. Discussion

This ex vivo investigation provides evidence supporting the effectiveness of a topical formulation composed of *Ophiopogon japonicus* extract, cyanocobalamin, and titrated *Centella asiatica* extract in modulating neurogenic inflammation and enhancing skin barrier function. The active ingredients in this formulation are known for their beneficial effects on sensitive and irritated skin and appear to exert a regulatory influence on TRPA1 expression, a key mediator involved in the pathophysiology of sensory disturbances such as pruritus and erythema.

TRPA1, a member of the transient receptor potential (TRP) ion channel family, has been implicated in the onset of neurogenic inflammation and cutaneous sensory symptoms. In this study, capsaicin was employed to induce TRPA1 expression, serving as a model for inflammatory skin activation. Following treatment with the formulation, a marked downregulation of TRPA1 expression was observed, suggesting a strong inhibitory effect. This effect was evident in a morphological assessment, including confocal microscopy imaging, which corroborated the suppression of TRPA1 signal.

The formulation's efficacy may be attributed to the combined effects of its constituents. Ophiopogon japonicus together with *Centella asiatica* and cyanocobalamin may help reduce TRPA1 activation by exerting anti-inflammatory and antioxidant effects, and by supporting skin barrier integrity. Together, these compounds appear to act on both inflammatory signaling pathways and the structural components of the skin barrier, offering dual therapeutic benefits.

Clinical evaluations further reinforced the ex vivo findings. Improvements were observed in parameters commonly associated with skin sensitivity, such as erythema, itching, and warmth sensation, as well as in measures of barrier function, including transepidermal water loss (TEWL). These outcomes are consistent with the known role of TRPA1 in mediating

vasodilation and sensory discomfort and suggest that its inhibition could translate into meaningful clinical improvements.

Overall, these findings highlight the potential of this multi-active formulation as a supportive treatment for individuals with hypersensitive skin. By targeting TRPA1-mediated inflammation and promoting barrier repair, it presents a comprehensive approach that addresses both the underlying biological mechanisms and the symptomatic manifestations of sensitive skin conditions.

5. Conclusion

A novel topical formulation has been developed comprising a rationally selected combination of *Ophiopogon japonicum* extract, cyanocobalamin, and titrated *Centella asiatica* extract. This formulation was specifically designed to modulate the expression of TRPA1—a pivotal mediator in cutaneous sensory transduction and neurogenic inflammation. Modulation of TRPA1 expression represents a promising therapeutic approach for the treatment of dermatological conditions associated with dysregulated sensory and inflammatory pathways.

This formulation adopts a multi-targeted therapeutic approach, addressing core mechanisms underlying skin reactivity, including oxidative stress, chronic inflammation, and barrier dysfunction as director indirect strategies to modulate TRPA1 activation.

Together, these actions position the formulation as a comprehensive therapeutic strategy for managing sensitive or inflammation-prone skin, with the potential to attenuate TRPA1-mediated pathways, enhance skin tolerance, and fortify the skin barrier against external irritants.

6. Bibliography

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