IFSCC 2025 full paper (IFSCC2025-1236)

Plant-derived Prinsepia CER-NP, New and Green Skincare Active in Soothing, Immune-regulating and Antioxidant

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1. Introduction

Human skin functions as a vital protective layer isolating the body from the external environment. A key contributor to this protective role is the lipid matrix within the stratum corneum (SC), the skin's outermost region[1]. The lipid composition of the SC is dominated by three principal categories: ceramides (CERs), cholesterol (CHOL), and free fatty acids (FFAs)[2]. When delving into the relative abundances of the various CER subclasses, CER-NP is the most abundant CER in human SC, constituting roughly 25–30 mol% of the total ceramide content [3], playing a central role in barrier homeostasis. There are many reports referred to the physiological and pathological importance of CERs and sphingolipids, which is indicative of that even small differences between CER species—i.e., in chain-length, hydroxylation status, and double bond number—has great impact on how they fulfill their species-specific functions[4]. However, there is few reference on what's impact on skin effect when multi-CER-NPs, particularly with specific ratio of different component, were employed.

Traditional CER-NP is characterized by a single structure, derivation from petrochemical materials, and its main efficacy being limited to barrier repair. With the improvement of consumers' health awareness and the popularization of environmental protection concepts, the market demand for personal care products has shifted from "quick results" to "green and sustainable.”

Plant-derived CER-NPs have emerged as a natural, safe, and sustainable upgraded products[5][6]. They are distinct from traditional ones, deriving from bio-based raw materials, with a 100% Natural Origin Index, fulfilling the highest standards of natural and organic cosmetics.

China boasts abundant and nutritious plant oils with diverse regional traits. Each region's unique climate and resources contribute to this richness and distinctness. Prinsepia utilis Royle oil is recorded in the "Southern Yunnan Materia Medica" more than 140 years earlier than the Compendium of Materia Medica. It is a rare woody oil plant with the effects of clearing heat

and detoxifying, activating blood, and removing stasis[7]. Prinsepia utilis Royle oil is native to southwest China, rich in a variety of unsaturated fatty acids. Its three main fatty acids of 1:1:0.8 are very close to the ratio of human fatty acid structure, which is biomimetic synthesis Ideal precursor for CER-NP.

In this study, we pioneered the development of plant-derived Prinsepia CER-NP - a multi-component complex engineered through small-molecule targeted modification technology. This innovative product successfully preserves the golden fatty acid ratio characteristic of native Prinsepia utilis Royle oil. Our discovery demonstrates that Prinsepia CER-NP with specific ratio of different CER-NP not only maintains the core efficacy profile of the original plant oil and ceramide, but achieves significant functional enhancements. It exhibits remarkable effect in soothing, immune-regulating and antioxidant. Notably, Prinsepia CER-NP demonstrates breakthrough efficacy in immune regulation, representing a new direction in the research of ceramides in immune barrier function.

2. Materials and Methods

2.1. Synthesis of Prinsepia CER-NP

Prinsepia utilis Royle oil was dissolved in ethanol and then heated to 80°C, by the subsequent addition of phytosphingosine. After stirred at 80°C for serval hours until completion of the reaction, the reaction system was cooled to room temperature. The reaction system was washed with deionized water and ethanol. The final drying process produced Prinsepia CER-NP as off-white powder.

2.2. IL-6 cytokine expression assay for soothing efficacy

RAW cells were planted in 96-well plates at a density of 1×10^4/well, placed in the incubator overnight, the supernatant was discarded for 24h, and 100μL of samples diluted by DMEM medium were added, and the negative control group was DMEM medium without drug, with 3 replicates in each group, incubated for 2h, and then added 10μg/mL LPS (lipopolysaccharide) to the model group and the experimental group and incubated together for 24h. At the end of the reaction, 50μL of cell supernatant was taken and the IL-6 ELISA kit was used to detect the expression of IL-6 gene in cells.

2.3 The receptor potential TRPV1 inhibition assay for soothing efficacy

HaCaT cells were planted in 96-well plates at a density of 1×10^4 cells/well, cultured and adhered at 37°C, 5% CO_2 overnight, and when the cells grew to 80% confluence, an appropriate concentration of capsaicin solution was added to simulate the activation state of the TRPV1 receptor. In capsaicin-treated cells, the supernatant was discarded, 100μL of the samples diluted in DMEM medium was added, trans-4-tert-butylcyclohexanol was set as a positive control, and after 24 hours of incubation, the upstream and downstream primers of the target gene were synthesized according to the TRPV1 primer design, and PCR amplification was performed. The amplification products were analyzed by agarose gel electrophoresis to observe the expression of the TRPV1 gene.

2.4 CAT and GSH enzyme activity were used to detect antioxidant efficacy

Fibroblast HFF-1 cells were planted in 96-well plates at a density of 1×10^4 cells/well, placed in the incubator overnight, the culture medium was removed after 24h, 2mL of PBS buffer solution was added to each well, DMEM medium containing different concentrations of samples was added, and the culture was continued for 24h. Place the plate on ice, add 1 mL of PBS buffer solution and 200μL of lysate to each well, and after thorough lysis, transfer the cell lysate to a 1.5mL centrifuge tube. The cells were further disrupted with an ultrasonic cell grinder for 1 min, centrifuged at 10000g for 3 min, 50μL of cell supernatant was taken and the kit was used to detect the expression of catalase CAT and glutathione GSH.

2.5 Human *β*-defensin 2 expression was used to detect immune efficacy

HaCaT cells were planted at a density of 1×10^4/well in 96-well plates and left overnight in an incubator. After 24h, the supernatant was discarded, 100μL of DMEM medium containing different concentrations of samples was added, 30mg/L quercetin was set as a positive control, and the medium was removed after 24h of incubation. The expression of human β-defensin 2 was detected by real-time PCR.

2.6 Raman spectroscopy was used to map penetration

The skin penetration behavior of Prinsepia CER-NP essential oil was evaluated in vivo using Labram Soleil high-resolution confocal Raman microscopy. Select the forearm as the test site. Before the measurement, gently cleanse the skin surface with purified water and then perform a 30-minute acclimatization cycle in a controlled environment. Use a keratometer to confirm skin barrier integrity. After uniform application of Prinsepia CER-NP essential oil, Raman spectra were obtained at predetermined time points (0 h, 0.5 h, 1 h, 1 h, 2 h, and 4 h). For each time point, five different locations within the test area were measured in triplicate to ensure data repeatability. At the end of the experiment, the spectral data were processed using LabSpec 6.0 software, and the characteristic vibration peaks of Prinsepia CER-NP essential oil were analyzed to determine the peak intensity, area, and full width of the maximum maximum (FWHM). Relative permeability (%) is calculated as:

$$RelativePermeation(\%) =$$

(Raman intensity on skin surface ÷ Raman intensity in skin)×100

2.7 Vivo test: 35 volunteers with sensitive skin

A total of 35 healthy men and women aged 20-40 years were selected, with dry facial features, weak barrier, and redness. After the subjects evenly applied Prinsepia CER-NP essential oil, the subjects were visited at the predetermined time points D0, D7, D14, and the area of the red area of the face was measured with VISIA CR, and the parameter values of the test area were significantly reduced, indicating that the sample had the effect of soothing redness, and the epidermal temperature was tested with a Tewameter thermal imager to verify that the sample had the effect of reducing the epidermal temperature.The study protocol received ethical approval from the Ellead Institutional Review Board (IRB approval number: EL-P-7400), and all participants provided written informed consent before enrollment.

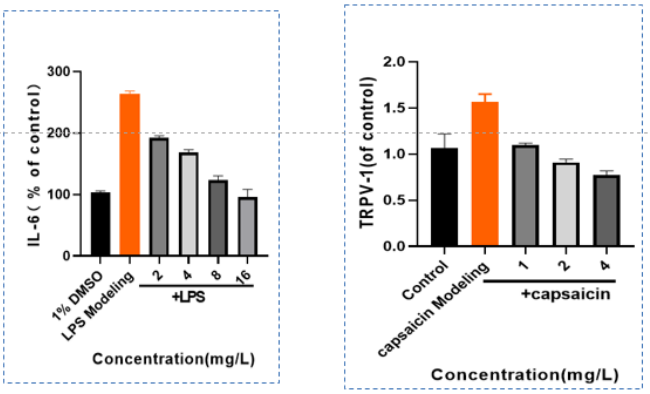
3. Results

3.1 Anti-inflammatory and Neurogenic Sensitivity

IL-6 inhibition: IL-6 is a core mediator of the pro-inflammatory response, and its overexpression is strongly associated with chronic inflammatory skin diseases such as atopic dermatitis. Prinsepia Cer-NP directly blocks the NF-κB signaling pathway and reduces the inflammatory cascade by inhibiting IL-6 secretion. The expression of IL-6 inflammatory factor was found to decrease by 63.72% at the concentration of 16 mg/L (**Figure 1.1a**).

Downregulation of TRPV1: TRPV1 is an ion channel that mediates neurogenic sensitivities such as skin burning and stinging. In this study, we found that Prinsepia CER-NP significantly reduced TRPV1 expression, and the mechanism may involve the regulation of transient receptor potential channels, thereby alleviating the "burning-itching" vicious cycle in sensitive skin. The expression of TRPV1 was found to decrease by 50.53% at the concentration of 4 mg/L (**Figure 1.1b**).

Prinsepia CER-NP realizes the dual regulation of inflammation and nerve, providing a solution for sensitive skin to suppress symptoms at the source.



(a) (b)

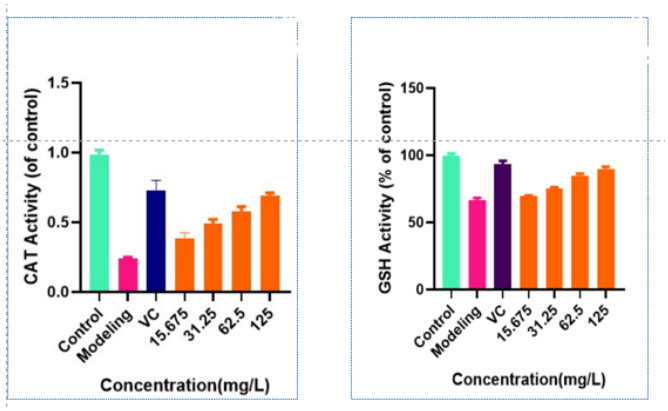
Figure 1.1 Soothing effect of Prinsepia CER-NP

3.2 Antioxidant Defense

CAT activity: Catalase (CAT) is a key enzyme in the neutralization of H_2O_2, and its activity surge indicates that Prinsepia Cer-NP is highly effective in scavenging reactive oxygen species (ROS) and preventing barrier damage caused by lipid peroxidation. At a concentration of 125 ppm, the activity of CAT increases by as much as 187.5% (**Figure 1.2a**).

GSH activity: Elevated levels of glutathione (GSH), the main antioxidant, further strengthen cellular reducing capacity and repair UV or pollution-induced oxidative damage. At a concentration of 125mg/L, the activity of GSH increases by 35.45% (**Figure 1.2b**).

Prinsepia CER-NP efficiently scavenges excess free radicals in the body by up-regulating the expression of antioxidant enzymes to protect against oxidative aging.



(a) (b)

Figure 1.2 Antioxidant effect of Prinsepia CER-NP

3.3 Immune Barrier Enhancement

Human β-defensin 2, a key antimicrobial peptide for skin innate immunity, is the first defensin found to be inducibly expressed. It is mainly derived from skin keratinocytes and mucosal epithelial cells. It has an important role in the intrinsic immune function of skin and mucosa and has strong killing activity against bacteria, fungi, viruses, and other microorganisms. When the concentration of Prinsepia CER-NP was 62.5mg/L, the expression of human β-defensin 2 increased by 82.78% (**Figure 1.3**), indicating that it can effectively regulate the skin immune process, defend against the colonization of pathogenic microorganisms by regulating the immune system and assisting in stimulating the body's immune response, and maintain the balance and stability of the skin microecology.

It's the first time that plant-derived CER-NP was found to regulate skin immune barrier. Prinsepia CER-NP, with golden ratio of different CER-NPs, promotes the synthesis of antimicrobial peptides, regulates the immune process of the body, and fills the scientific research gap of ceramide in the immune barrier.

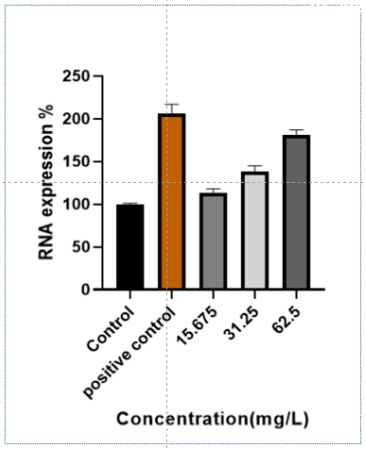


Figure 1.3 Immune barrier-repairing effect of Prinsepia CER-NP

3.4 Efficient Penetration

The intrinsic Raman spectrum of human in vivo skin revealed characteristic vibrational peaks at 943 cm^{-1}, 1275 cm^{-1}, 1455 cm^{-1}, 1655 cm^{-1}, 2846 cm^{-1}, 2883 cm^{-1}, 2934 cm^{-1}, and 3226 cm^{-1} (**Figure 2.1**). These spectral signatures correspond to key biomolecular components, including Amino acids (proline, hydroxyproline, glycine),Nucleic acids,Structural proteins (elastin, actin, collagen, keratin)，and Intercellular lipids (**Table 1**).

Table 1. Attribution of Raman characteristic peaks in human in vivo skin and their attribution with representative components

Peak position (cm^{-1})	Vibration mode	Main Representative Ingredients
943	N(C-C) backbone, collagen backbone	Proline, Hydroxyproline
1275	CN absorption band (amide I band)	Glycine backbone, proline, nucleic acid
1455	C-H bending modes of proteins (CH2 stretching/CH3 asymmetric deformation)	Structural proteins, elastin
1655	vC=O stretching vibrations (amide I band, inclusion-folding, refolding and random curling)	Actin, Collagen, Keratin
2846	Asymmetric stretching of CH2	Lipids
2883	Symmetric stretching of CH2	Lipids
2934	Asymmetric stretching of CH3	Lipids and proteins

This comprehensive spectral profiling established foundational benchmarks for evaluating cutaneous biochemistry prior to Prinsepia CER-NP administration. Subsequent Raman spectroscopic mapping elucidated the compound's permeation dynamics, revealing its time-dependent accumulation within the stratum corneum. The unique spectral signatures of both native skin constituents and the applied Prinsepia CER-NP formulation enabled precise discrimination between endogenous biological components and the exogenous complex, offering critical validation of its cutaneous delivery performance.

Structural characterization of Prinsepia CER-NP identified diagnostic vibrational modes between 876-3333 cm^{-1} (**Figure 2.2**), confirming molecular integrity through characteristic bond oscillations. Depth-resolved Raman imaging (**Figure 2.3**) revealed the compound's dynamic penetration kinetics. While initial measurements at 0 h showed no Prinsepia CER-NP in the stratum corneum, progressive permeation was observed at 0.5 h (1.12%), escalating to 3.07% (1 h), 6.85% (2 h), and 8.23% (4 h). the maximum permeability depth is 70 μm(**Table 2**). Raman spectroscopy confirms that Prinsepia CER -NP can penetrate into the granular layer of the epidermis and deliver targeted delivery to the active epidermal layer.

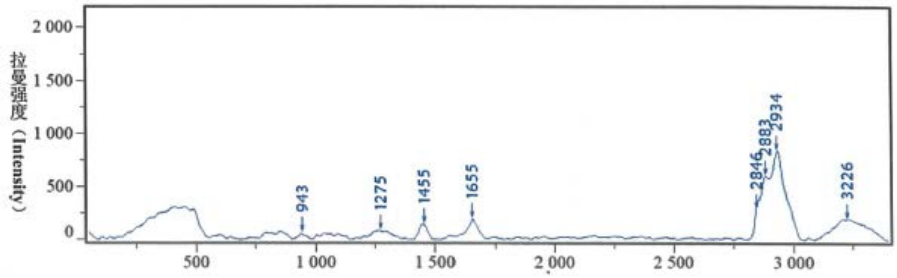


Figure 2.1 Raman spectrum of human in vivo skin

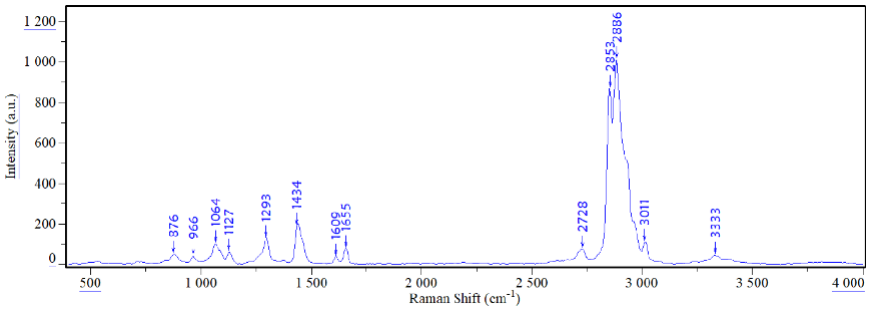


Figure 2.2 Raman spectrum of Prinsepia CER -NP

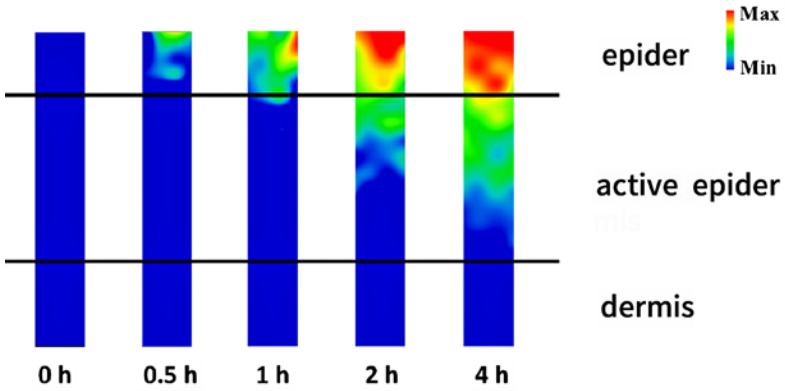


Figure 2.3 Raman spectroscopy of Prinsepia CER -NP-containing oil. Depth penetration is indicated by color scale from dark blue (no penetration) to dark red (high penetration)

Table 2. Prinsepia CER -NP penetration depth

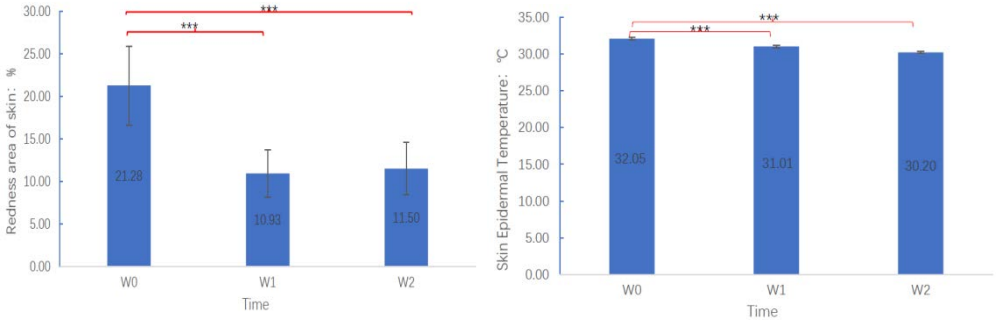
Prinsepia CER -NP use time (h)	Maximum depth of penetration (μm)
0.5	20
1	25
2	55
4	70

3.5 Vivo Test Verification

the 14-day clinical study systematically evaluated the lowering the skin temperature, and soothing effects of Prinsepia CER -NP. In the first week of use, compared with before use, the

proportion of red zone area was significantly reduced by 48.64% (p<0.001); in the second week of use, the proportion of red zone area was significantly reduced by 48.64% (p<0.001)(**Figure 3a**),confirming its efficacy in soothing.At the same time, epidermal temperature was significantly reduced by 3.24% (p<0.001) in one week ; Epidermal temperature was significantly reduced by 5.77% (P<0.001) in the second week, demonstrating its long-term cooling and sedative effects(**Figure 3b**).

Prinsepia CER-NP calms the skin by reducing the size of red zones and lowering the skin temperature, providing a new way of thinking about high-efficacy sensitive skin care (e.g., post-medical).



(a) (b)

Figure 3 Soothing effect of Prinsepia CER -NP-containing oil.

4. Discussion

This study introduces plant-derived Prinsepia CER-NP, a novel green CER, using natural Prinsepia utilis Royle oil and phytosphingosine as starting materials. We developed a multi-component CER-NP complex, whose proportion basically follows the golden proportion of fatty acids in natural oil, benefiting from precise targeted modification of small molecules. Prinsepia CER-NP can not only enhance skin barrier function but also exhibit unprecedented anti-inflammatory, antioxidant, and immune-modulating properties. In vitro trials demonstrated significant reductions in IL-6 and TRPV1 expression, alongside elevated CAT and GSH activity. Raman spectroscopy confirmed efficient epidermal penetration, while clinical studies revealed a 48.64% reduction in red zone in one week and a 5.77% skin temperature drop within 2 weeks. Collectively, it is suitable for high-end applications such as anti-oxidation and immune skin care of sensitive skin.

5. Conclusion

This study establishes plant-derived Prinsepia CER-NP as a groundbreaking advancement in ceramide science. By synergizing the unique phytochemistry of Prinsepia utilis Royle oil with green synthesis technology, we have engineered a novel complex that simultaneously addresses three critical challenges: barrier dysfunction, neurogenic inflammation, and oxidative

stress. As the first CER-NP demonstrating immunomodulatory activity, this innovation bridges the gap between barrier repair and immune regulation. It opens up a new way for sensitive skin care. The underlying relationship between Prinsepia CER-NP and its excellent performance in sensitive and aging skin care certainly deserves to be further explored.

Plant-derived ceramide compliance with ISO 16128 standards for natural cosmetics further solidifies its commercial viability, aligning with global demands for sustainable, high-performance skincare actives that integrate green chemistry with precision efficacy.

Notably, this work expands the functional paradigm of ceramides, particularly for CER-NP—the most abundant ceramide subclass in human skin. By leveraging China's vast botanical diversity and regionally unique plant resources, we have demonstrated the feasibility of developing biomimetic, plant-derived ceramides with tailored efficacy. Looking ahead, the exploration of diverse phytoceramide sources, especially those with distinct fatty acid profiles and ratios, promises to unlock further differentiated efficacy. Future research should focus on elucidating structure-activity relationships between plant-derived ceramide and targeted skin benefits, paving the way for a new generation of geographically inspired products.

In conclusion, Prinsepia CER-NP exemplifies how traditional botanical wisdom, when integrated with modern green technology, can yield scientifically validated solutions that meet both ecological and clinical imperatives. This work not only advances ceramide science but also sets a precedent for harnessing regional biodiversity to address global skincare challenges.

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