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# The Physicochemical Properties, Antioxidant Potential, and Skin Penetration of Pterostilbene-Loaded NLCs

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*HIGHLIGHTS*

- Pterostilbene was successfully encapsulated into nanostructured lipid carriers (PT-NLCs) with high encapsulation efficiency (~99.98%) and nanoscale particle size (~88.6 nm)
- PT-NLCs increased water solubility by approximately 5,506% compared with raw pterostilbene and enhanced skin penetration by about 360% relative to the free compound
- PT-NLCs exhibited ~34% higher antioxidant activity than raw pterostilbene and showed strong potential as a topical delivery system to protect skin cells from oxidative stress

**ABSTRACT**

Pterostilbene is a naturally occurring polyphenolic compound known for its antioxidant activity and potential use in topical skin care formulations. However, its poor water solubility and limited skin penetration reduce its therapeutic effectiveness. In this study, pterostilbene-loaded nanostructured lipid carriers (PT-NLCs) were developed and evaluated as a topical delivery system to enhance its stability and dermal performance. The PT-NLCs showed a mean particle size of 78–99 nm (88.57 ± 10.60 nm), a polydispersity index of 0.284, and an encapsulation efficiency of 99.98%, indicating a stable nanosystem. The NLC formulation increased apparent water solubility by over 5,000-fold and improved skin penetration by approximately 360% compared with raw pterostilbene. PT-NLCs also demonstrated enhanced antioxidant activity, with about a 34% increase in radical scavenging capacity in the DPPH assay and improved protection of skin cells against oxidative stress while maintaining good cytocompatibility. These findings demonstrate that NLC-based encapsulation effectively improves the solubility, dermal penetration, and antioxidant efficacy of pterostilbene, highlighting its potential as a promising ingredient for topical skin treatment applications.

## INTRODUCTION

The human skin is a complex organ composed of diverse proteins and cell types, functioning as a barrier while also playing essential roles in repair, thermoregulation, and immune recognition (Barbieri et al., 2014). As a dynamic interface continuously exposed to environmental stressors, the skin is susceptible to conditions that negatively affect both appearance and health, including acne, clogged pores, post-acne scarring, and sensitivity caused by improper product use, exposure to harmful substances, and inadequate makeup removal (Chu et al., 2022). Effective skin treatment strategies aim to restore barrier integrity, reduce inflammation, and support cellular health through topical and systemic interventions, including the use of bioactive natural compounds such as stilbenoids.

Stilbenoids are plant-derived phenolic compounds characterized by a C6–C2–C6 backbone structure and belong to the phenylpropanoid family (Akinwumi et al., 2018). These phytoalexins are synthesized by plants in response to environmental stressors such as ultraviolet radiation and microbial infection, contributing to their strong biological activity (Bo et al., 2022). Extensive research has demonstrated that stilbenoids possess antioxidant, anti-inflammatory, and antimicrobial properties, making them attractive candidates for dermatological applications (Oh et al., 2021). In cosmetic science, stilbenoids have been associated with multiple skin benefits, including whitening effects, wrinkle reduction, improved elasticity, enhanced moisturization, and protection against oxidative stress-induced aging (Park et al., 2020).

Pterostilbene, a naturally occurring dimethylated derivative of resveratrol with the molecular formula $C_{16}H_{16}O_3$, has gained attention due to its favorable physicochemical and biological properties. Structurally, pterostilbene contains two methoxy groups and one hydroxyl group, which increase its lipophilicity compared to resveratrol, thereby enhancing cellular uptake, bioavailability, and metabolic stability (Lin et al., 2020). Notably, pterostilbene exhibits a significantly longer half-life (105 min) than resveratrol (14 min), suggesting prolonged biological activity. Although pterostilbene has been extensively marketed as an oral supplement, its application as a cosmetic ingredient remains relatively underexplored despite growing evidence of its ability to protect keratinocytes from particulate matter-induced damage, reduce oxidative stress and inflammation, delay skin aging, and improve skin moisturization (Teng et al., 2021).

In topical skin treatment, several antioxidant compounds such as resveratrol, vitamin C (ascorbic acid), vitamin E (tocopherol), ferulic acid, and niacinamide are widely used to combat oxidative stress, inflammation, and photoaging. While these agents demonstrate proven efficacy and are commercially well established, many suffer from formulation-related limitations, including poor chemical stability, rapid oxidation, limited skin penetration, or reduced bioavailability (Yang et al., 2024). For example, resveratrol is highly unstable under light and oxygen exposure, while ascorbic acid degrades rapidly in aqueous environments, necessitating complex formulation strategies. Compared to these alternatives, pterostilbene offers superior lipophilicity, enhanced stability, and longer biological half-life, which collectively provide a strong advantage for sustained topical activity and improved dermal delivery.

Despite its promising dermatological benefits, pterostilbene also presents formulation challenges that limit its direct topical application. Its extremely low water solubility (approximately 0.0180 mg/g) can hinder absorption and reduce therapeutic efficacy when applied to the skin (Haq et al., 2023). Additionally, pterostilbene is susceptible to degradation upon exposure to light, air, and ultraviolet radiation, which may compromise antioxidant performance and shorten product shelf life (Waszczuk et al., 2020; Yang et al., 2024). To overcome these limitations, nano-based delivery systems have been increasingly explored to enhance the solubility, stability, and bioavailability of poorly water-soluble bioactives.

Nanostructured lipid carriers (NLCs) are advanced lipid-based nanodelivery systems composed of a mixture of solid and liquid lipids, forming stable spherical matrices capable of efficiently encapsulating lipophilic compounds (Garg et al., 2022). NLCs have gained considerable interest in topical formulations due to their ability to enhance drug loading, improve physicochemical stability, promote deeper skin penetration, and provide controlled release while maintaining excellent biocompatibility and low toxicity (Fitriani et al., 2024; Gomaa et al., 2022). Compared to conventional lipid nanoparticles, NLCs offer superior encapsulation efficiency, longer shelf life, and scalable manufacturing processes suitable for industrial production (Chauhan et al., 2020).

Although various nanoformulation strategies have been investigated for antioxidant delivery in skincare, existing studies on pterostilbene-loaded nanocarriers remain limited and often focus primarily on oral or systemic applications, with insufficient emphasis on topical performance, skin penetration behavior, and safety evaluation at the cellular level. In particular, systematic investigations addressing the combined improvement of water solubility, dermal permeability, and cytocompatibility of pterostilbene using NLC-based systems are still lacking. Therefore, this study aims to develop pterostilbene-loaded nanostructured lipid carriers (PT-NLCs) to enhance skin permeability and aqueous solubility, while comprehensively evaluating their physicochemical characteristics and safety through cell viability assessment.

## MATERIALS AND METHODS

### Materials

Pterostilbene (PT, ≥98% purity), hydroxypropyl methylcellulose (HPMC), Tween 80, Span 80, Olivem 300, caprylic triglycerides, squalene, diethylene glycol monoethyl ether (Transcutol® P), methanol, acetonitrile, potassium dihydrogen phosphate ($KH_2PO_4$), and dipotassium phosphate ($K_2HPO_4$) were of analytical or pharmaceutical grade and used as received. PT and HPMC were obtained from Huanqi International Trading Co., Ltd. (Taiwan). Tween 80 and Span 80 were purchased from First Chemical Works (Taiwan). Olivem 300 was supplied by Taiwan High and Better Corp. (Taiwan), while caprylic triglycerides and squalene were obtained from HonorChem Co., Ltd. (Taiwan). Methanol and acetonitrile were purchased from Fisher Scientific (Seoul, Korea). $KH_2PO_4$ and $K_2HPO_4$ were obtained from Ferak (Berlin, Germany). The HaCaT human keratinocyte cell line was sourced from the Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna (Brescia, Italy). Dulbecco's modified Eagle's medium (DMEM) was purchased from Himedia Laboratories (Mumbai, India), fetal bovine serum (FBS) from Thermo Fisher Scientific (Waltham, MA, USA), and MTT reagent from MDBio (Taipei, Taiwan).

### Methods

#### Preparation of PT-NLCs

The composition of PT-NLCs is shown in **Table 1**. The formulation was divided into Phase A (lipid and surfactant phase) and Phase B (drug and polymer phase). Phase B is highlighted in red in **Table 1** to indicate the drug-loading phase, as it contains pterostilbene and hydrophilic excipients that are critical for solubilization, dispersion, and subsequent encapsulation efficiency.

For Phase A, Tween 80 (3.24 g), Span 80 (1.12 g), Olivem 300 (0.36 g), caprylic triglycerides (2.72 g), and squalene (0.90 g) were weighed and mixed using a magnetic stirrer until a homogeneous lipid phase was obtained. For Phase B, pterostilbene (0.40 g) was dissolved in Transcutol® P (1.16 g) under heating. The solution was heated to 200 °C for 10 min to ensure complete dissolution of pterostilbene, which is poorly soluble at lower temperatures. Although high temperatures may raise concerns regarding thermal

degradation, the short exposure time and the stabilizing effect of Transcutol® P minimized degradation risk. HPMC (0.20 g, 3% w/w) was then added to Phase B and stirred for an additional 10 min to obtain a uniform dispersion.

Phase B was slowly added to Phase A under continuous stirring, followed by high-speed homogenization at 22,000 rpm for 10 min. The resulting emulsion was further processed using a probe sonicator homogenizer for 10 min to reduce particle size and obtain stable PT-NLCs.

**Table 1.** Formula for Pterostilbene NLCs

| | Ingredients | Content (%, w/w) | /20g |
|---|---|---|---|
| **Phase A** | Tween 80 | 16.2 | 3.24g |
| | Span 80 | 5.6 | 1.12g |
| | Olivem 300 | 1.8 | 0.36g |
| | Caprylic triglycerides | 13.6 | 2.72g |
| | Squalene | 4.5 | 0.9g |
| **Phase B** | Diethylene glycol monoethyl ether | 5.8 | 1.16g |
| | Pterostilbene | 2.0 | 0.4g |
| | 3% HPMC | 1.0 | 0.2g |

**Particle size measurement**

0.1 g of pterostilbene NLCs sample was diluted into 2 mL of double-distilled water (DDH2O). Then, 100 uL of the solution was diluted in 1900 uL of DDH2O to create a 20-fold dilution. The solution was resuspended and immediately placed in a cuvette to determine the average particle size using a particle size analyzer (ELSZ-2000; Otsuka Electronics, Osaka, Japan).

**HPLC analysis of PT**

The standard curve for PT was generated using high-performance liquid chromatography (HPLC). The HPLC apparatus (LaChrom Elite L-2000, Hitachi, Tokyo, Japan) has an L-2130 pump, an L-2200 autosampler, and an L-2420 (UV-vis) detector connected to a Mightysil RP-18 GP column (250 × 4.6 mm, 5 µm; Kanto Chemical Co., Inc., Tokyo, Japan). The mobile phase was composed of acetonitrile and water at a constant ratio of 75:25 (v/v). The system's flow rate was set to 1 mL/min, the injection volume was 10 µL, and the wavelength was monitored at 360 nm. PT was dissolved in methanol and diluted to nine concentrations (0.005-100 µg/mL) to create the standard curve. The retention time peak for PT was reported at 4.2 minutes.

**Water solubility**

Pure PT (1 mg) and PT-NLCs (1 mg PT) were dissolved in 1 mL of water and shaken (Vortex-Gene 2, Scientific Industries, Bohemia, NY, USA) for 10 minutes, respectively. Each sample was filtered with a 0.45 µm syringe filter (13 mm Acrodisc® syringe filters with GHP membrane, Pall Corporation, Port Washington,

NY, USA) and diluted 10-fold with water before HPLC analysis. The PT concentration was calculated using the PT standard curve.

**Yield and encapsulation efficiency**

First, PT-NLCs (containing 1 mg PT) were weighed, inserted in a 1.5 mL tube with 1 mL methanol, and shaken for 10 minutes. To determine the yield, the PT concentration in each sample was calculated using the previously mentioned HPLC analytical procedure. To determine encapsulation efficiency, PT-NLCs containing 1 mg MYR were weighed and dissolved in 1 mL of distilled water. Next, 500 µL of each sample was placed in a high-speed centrifugal filter device (Nanosep® Centrifugal Devices with Omega™ Membrane molecular weight 10,000, Pall Corporation, Port Washington, NY, USA) and centrifuged at 10,000 rpm (Centrifuge 5430 R; Eppendorf, Hamburg, Germany) for 10 minutes. The PT concentration in each sample was determined using the HPLC analysis method described above. The yield and encapsulation efficiency of PT-NLCs were calculated using **Equations 1** and **2**, respectively.

$$Yield\ (\%) = \frac{actual\ amount}{theoretical\ amount} \times 100\%$$

**Equation 1.** Yield (%) Equation

$$Encapsulation\ efficiency\ (\%) = \frac{Weight\ of\ unencapsulation\ part - weight\ of\ empty\ tube}{actual\ weight} \times 100\%$$

**Equation 2.** Encapsulation Efficiency Equation

**In vitro skin penetration assay**

An in vitro skin penetration experiment was performed using a modified approach from the European Cosmetic and Perfumery Association (COLIPA). Pig skin was purchased from a local market and sliced into a 4 cm2 patch (2 cm x 2 cm). The skin penetration of PT via pig skin was measured using the Franz diffusion cell (FDC) technique. The pig's skin was inserted between the donor (upper) and receptor (lower) chambers of the FDC system. The receptor chambers were first filled with a buffer solution (0.14 M NaCl, 2 mM K2HPO4, 0.4 mM KH2PO4, and pH 7.4), followed by a magnetic stirring bar at the bottom. The FDC system was maintained at 32 °C under the circulating water system and stirred at 600 rpm. Next, 200 µL of 1 mg/mL of PT in water and PT-NLCs in water (containing 1 mg PT) were placed into the pig's skin. The sample intervals for topical administration were set at 30 minutes and one hour. Following that, the skin sample's stratum corneum was obtained by stripping 15 times with 3 M Transpore tape. The epidermal and dermal layers were separated using a knife and cut into small pieces. Each sample was then placed in a methanol-filled tube, and the MYR was extracted with a sonicator for 1 hour. The PT content of each sample was determined using the HPLC approach described above.

**DPPH free radical scavenging activity**

The DPPH free radical scavenging activity was used to assess the antioxidant activity of substances. Raw PT in water, raw PT in ethanol, PT-NLCs in water, and vitamin C in water served as positive controls. To begin, 100 µL of 200 µM DPPH free radical solution and 100 µL of each sample were pipetted into each well of the 96-well plate, then shaken for 30 minutes. A microplate spectrophotometer with a 517 nm wavelength was used to detect absorbance. The SC50 of each sample was calculated using **Equation 3**.

$$SC50 = \left(1 - \frac{sample517nm}{blank517nm}\right) \times 100\%$$

**Equation 3.** SC50 Equation

**Cell culture and cell safety assay**

The HaCaT keratinocytes were grown in DMEM media (with 10% FBS and 1% PSA) in an incubator at 37 °C with 5% CO2 (Thermo Fisher Scientific, Waltham, MA, USA). To evaluate the cell safety of PT and PT-NLCs, 100 µL of media with 1 × 104 HaCaT cells was seeded into each well of a 96-well plate for 24 hours. After removing the culture media from each well, varied concentrations (5-40 µM) of PT in PBS, PT in DMSO, and PT-NLCs in PBS were added to the wells with serum-free DMEM medium for 24 hours. After 24 hours, the culture media was removed, and the cells were rinsed with 100 µL PBS. To each well, 150 µL of MTT (0.5 mg/mL) solution was added, and the plates were incubated for 3 h. Then, the MTT solution of each well was removed, and 100 µL of DMSO was added to dissolve the purple formazan crystals. A microplate spectrophotometer at 550 nm was used to measure absorbance.

**Statistical analysis**

All experiments were performed in triplicate, and results are presented as mean ± standard deviation. Statistical significance between groups was analyzed using Student's t-test or one-way analysis of variance (ANOVA), followed by post hoc testing where appropriate. A p-value < 0.05 was considered statistically significant.

## RESULTS

**Yield, encapsulation efficiency, and water solubility of PT and PT-NLCs**

To evaluate the formulation performance of pterostilbene-loaded nanostructured lipid carriers, yield, encapsulation efficiency, and aqueous solubility were determined and compared with raw pterostilbene. These parameters are critical indicators of formulation efficiency and suitability for topical delivery.

As summarized in **Table 2** below, PT-NLCs exhibited an excellent formulation yield exceeding 99.9%, indicating minimal material loss during preparation. The encapsulation efficiency of PT within the NLC system reached 99.98 ± 0.01%, demonstrating highly effective incorporation of pterostilbene into the lipid matrix. In contrast, raw pterostilbene displayed extremely poor water solubility, measured at only 0.19 ± 0.01 µg/mL. Encapsulation into NLCs resulted in a dramatic and statistically significant increase in aqueous solubility to 1046.23 ± 78.78 µg/mL ($p < 0.05$), representing an improvement of more than 5,000-fold, calculated as 1046.23 µg/mL (PT-NLCs) divided by 0.19 µg/mL (raw PT). These findings confirm that NLC encapsulation effectively overcomes the inherent solubility limitations of pterostilbene.

**Table 2.** The yield, encapsulation efficiency, and water solubility of PT-NLCs

| Formulation | Yield (%) | Encapsulation efficiency (%) | Water solubility (µg/ml) |
|---|---|---|---|
| PT in water | - | - | 0.19 ± 0.01 |
| PT-NLCs | >99.9 | 99.98 ± 0.01 | 1046.23 ± 78.78* |

Values are mean ± SD (n=3); *$p < 0.05$ indicated a statistically significant difference compared with PT in water.

**Particle size and polydispersity index of PT and PT-NLCs**

Particle size and polydispersity index (PDI) were measured to assess the dispersion quality and nanoscale characteristics of the PT-NLC formulation, which are essential for stability and skin penetration.
As shown in **Table 3**, raw pterostilbene exhibited an extremely large particle size (33,989.1 nm) and a very high PDI value (7.631), indicating severe aggregation and poor dispersion in aqueous media. In contrast, PT-NLCs demonstrated a significantly reduced mean particle size of 88.57 ± 10.60 nm with a PDI of 0.284,

reflecting a narrow size distribution and high colloidal stability. These results confirm that encapsulation into NLCs successfully converts pterostilbene into a uniform nanoscale formulation suitable for topical application.

**Table 3.** Particle size of PT and PT-NLCs

| | Particle size (nm) | Polydispersity index (PDI) |
|---|---|---|
| PT | 33989.1 | 7.631 |
| PT-NLCs | 88.57 ± 10.6 | 0.284 |

**Antioxidant activity of PT and PT-NLCs by DPPH assay analysis**

The antioxidant capacity of PT and PT-NLCs was evaluated using the DPPH free radical scavenging assay to determine whether nanoencapsulation enhances functional activity.

As presented in **Table 4** and **Figure 1**, raw PT dispersed in water exhibited no detectable scavenging activity due to its poor solubility. PT dissolved in ethanol showed measurable antioxidant activity, with an $SC_{50}$ value of 33.04 ± 1.48 µg/mL. Notably, PT-NLCs dispersed in water demonstrated significantly enhanced antioxidant activity, with a lower $SC_{50}$ value of 21.76 ± 2.07 µg/mL ($p < 0.05$), indicating stronger free radical scavenging capability than PT in ethanol. Vitamin C, used as a positive control, showed the highest activity with an $SC_{50}$ of 11.83 ± 1.02 µg/mL. These results indicate that NLC encapsulation not only improves PT solubility but also enhances its antioxidant effectiveness in aqueous environments.

**Table 4.** The free radical scavenging effect of PT and PT NLC

| Samples | SC50 (µg/mL)[a] |
|---|---|
| | DPPH free radical |
| PT in water | NE[c] |
| PT in ethanol | 33.04 ± 1.48 |
| PT-NLC in water | 21.76 ± 2.07[*#] |
| Vitamin C[b] | 11.83 ± 1.02 |

Values are mean ± SD (n=3); a SC50 indicates that the extract scavenges 50 % of DPPH free radicals; b Vitamin C was used as the positive control; c NE indicated no effect; * $p < 0.05$ indicated a statistically significant difference compared with PT in ethanol; # $p < 0.05$ indicated a statistically significant difference compared with Vitamin C.

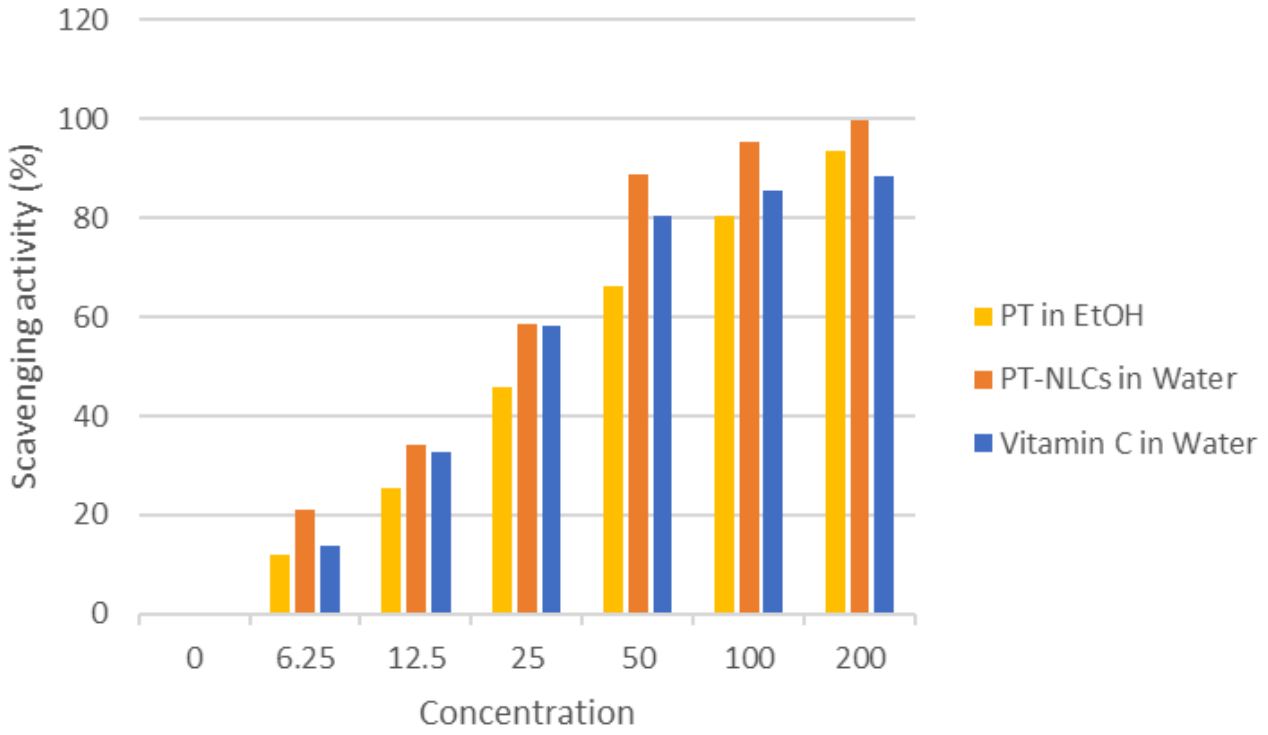


**Figure 1.** DPPH free radical scavenging activity of PT in ethanol, PT-NLCs in water, and vitamin C. Data are presented as mean ± SD (n = 3). Error bars represent standard deviation. Statistical significance is indicated by $p < 0.05$.

**In vitro skin penetration of PT and PT-NLCs**

The ability of PT-NLCs to enhance dermal delivery was evaluated using an in vitro Franz diffusion cell model with porcine skin. This skin-layer separation method has been widely reported to reliably differentiate the stratum corneum, epidermis, and dermis in in vitro permeation studies, minimizing cross-layer contamination. Skin layers were separated by standardized tape stripping for the stratum corneum, followed by careful mechanical separation of the epidermis and dermis to ensure consistent layer isolation.

At both 0.5 h and 1 h (**Table 5**), raw PT exhibited limited penetration, with relatively low concentrations detected in the stratum corneum and dermis. In contrast, PT-NLCs showed markedly higher PT deposition across all skin layers. At 0.5 h, PT-NLCs increased PT accumulation in the stratum corneum (43.01 ± 13.00 µg/mL) and dermis (22.09 ± 8.16 µg/mL) compared to raw PT. A similar trend was observed at 1 h, where PT-NLCs maintained substantially higher dermal penetration. These findings demonstrate that NLC encapsulation significantly enhances the transdermal delivery and retention of pterostilbene.

**Table 5.** Skin penetration of PT and PT-NLCs at 0.5 h and 1 h

| PT conc. (µg/ml) | Interval timing | Stratum corneum | Epidermis | Dermis |
|---|---|---|---|---|
| PT in water | 30 minutes | 18.68 ± 7 | 24.38 ± 5.15 | 9.26 ±1.15 |
| PT-NLCs in water | | 43.01 ± 13 | 32.61 ± 10.56 | 22.09 ± 8.16 |
| PT in water | 1 hour | 11.96 ± 4 | 41 ± 3.25 | 5.39 ± 3.23 |
| PT-NLCs in water | | 40.62 ± 3.3 | 41 ± 8.88 | 24.99 ± 2.9 |

**Cell safety assessment of PT and PT-NLCs**

The cytocompatibility of PT and PT-NLCs was assessed using HaCaT human keratinocytes to evaluate formulation safety for topical application.

As shown in **Figure 3**, PT-NLCs in PBS and PT dissolved in DMSO maintained cell viability above 80% across concentrations ranging from 5 to 20 µM. In contrast, PT dispersed in PBS showed minimal effect due to its limited solubility. These results indicate that PT-NLCs exhibit good cellular compatibility and are safe for use in skin-related applications at the tested concentrations.

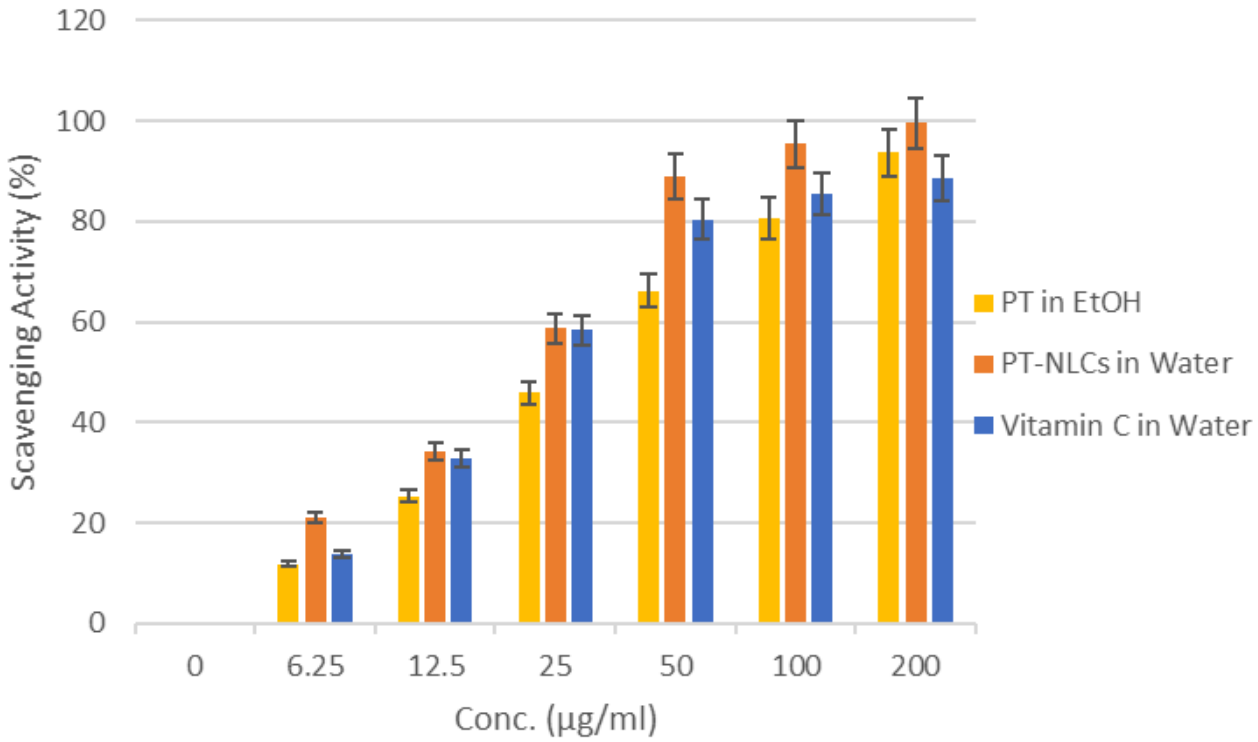


**Figure 3.** Cell viability of HaCaT keratinocytes treated with PT and PT-NLCs. Data are expressed as mean ± SD (n = 3). Error bars indicate standard deviation

## DISCUSSION

Nanostructured lipid carriers (NLCs) were employed in this study to overcome the intrinsic formulation limitations of pterostilbene, particularly its extremely low aqueous solubility, which restricts topical bioavailability and clinical applicability (Li et al., 2024). The NLC system provides a heterogeneous lipid matrix composed of solid and liquid lipids, creating structural imperfections that enhance drug accommodation and retention (Chauhan et al., 2020; Mahor et al., 2023). In this formulation, pterostilbene is distributed within both lipid phases rather than crystallizing on the particle surface, which contributes to the high encapsulation efficiency and formulation yield observed. The presence of surfactants further stabilizes the nanocarriers by reducing interfacial tension and preventing drug expulsion during storage and application (Khan et al., 2022).

The significant improvement in aqueous solubility achieved through NLC encapsulation can be attributed to multiple synergistic mechanisms. First, nanoscale particle size dramatically increases the effective surface area available for interaction with the aqueous environment, thereby enhancing apparent solubility (Danaei et al., 2018). Second, molecular dispersion or amorphization of pterostilbene within the lipid matrix reduces crystal lattice energy compared with the raw crystalline drug (Mahor et al., 2023).

Compared with other nanocarrier systems used for hydrophobic polyphenols, such as polymeric nanoparticles and nanoemulsions, NLCs often demonstrate superior solubilization capacity. Polymeric nanoparticles and nanoemulsions typically enhance solubility by approximately $10^2$–$10^3$ fold, whereas lipid-based nanocarriers frequently exceed these values due to higher drug loading and lipid–drug affinity (Naseri et al., 2015; Khan et al., 2022).

Particle size reduction plays a critical role in formulation stability and skin delivery performance. Transitioning pterostilbene from the micrometer scale to a nanoscale carrier enhances kinetic solubility and establishes a higher concentration gradient across the stratum corneum, which is the primary driving force for passive skin diffusion (Chaiprateep et al., 2023). Nanoparticles below 100 nm have been reported to preferentially accumulate in hair follicles and intercellular lipid pathways, enabling deeper penetration into viable epidermal and dermal layers (Adib et al., 2016). In addition, the low polydispersity index obtained in this study indicates a uniform particle population, which is essential for reproducible skin permeation and colloidal stability (Ohenoja et al., 2014; Danaei et al., 2018).

Encapsulation of pterostilbene into NLCs also enhanced antioxidant activity, as demonstrated by improved DPPH radical-scavenging performance. The DPPH assay is widely used to evaluate antioxidant capacity through proton or electron donation mechanisms (Fatiha & Abdelkader, 2019). While raw pterostilbene exhibits strong intrinsic antioxidant properties, its poor solubility in aqueous media severely limits its measurable activity in water-based systems (Yang et al., 2024). Nanoencapsulation improves the dispersion and accessibility of pterostilbene, enabling more effective interaction with DPPH radicals. Although vitamin C demonstrated superior antioxidant activity, ascorbic acid is known to exhibit poor stability and rapid degradation in topical formulations (Chen et al., 2016; Lespade, 2017). In contrast, lipid-based nanocarriers provide a protective environment that supports sustained antioxidant performance.

Enhanced skin penetration of PT-NLCs further supports the suitability of this delivery system for topical applications. Pig skin was selected as the penetration model due to its structural and permeability similarity to human skin (Herbig et al., 2015). Compared with raw pterostilbene, PT-NLCs demonstrated increased accumulation across the stratum corneum, epidermal, and dermal layers. This behavior is consistent with previous reports indicating that lipid nanoparticles interact with and partially fluidize stratum corneum lipids, thereby enhancing drug permeation (Naseri et al., 2015). Additionally, the occlusive properties of lipid nanoparticles may increase skin hydration, indirectly promoting penetration by loosening the corneocyte packing (Chauhan et al., 2020).

Cytocompatibility assessment using HaCaT keratinocytes revealed a concentration-dependent reduction in cell viability for PT-NLCs compared with raw pterostilbene. This effect is likely associated with enhanced cellular uptake resulting from reduced particle size and increased intracellular delivery of pterostilbene (Hoshyar et al., 2016). Moreover, NLCs may provide sustained drug release, prolonging intracellular exposure and amplifying biological effects (Zhang et al., 2019). While this indicates improved bioavailability, it also underscores the importance of dose optimization to maintain cellular safety in topical applications.

Several limitations of this study should be acknowledged. First, all experiments were conducted using in vitro and ex vivo models, and in vivo skin penetration and safety were not evaluated. Second, the long-term physical and chemical stability of PT-NLCs under different storage conditions was not assessed, which is essential for product development and commercialization. Finally, although antioxidant activity and cytocompatibility were demonstrated, additional biological endpoints such as anti-inflammatory,

photoprotective, or anti-aging effects should be explored to fully establish the therapeutic potential of PT-NLCs.

Overall, these findings demonstrate that nanostructured lipid carriers represent a robust and effective platform for enhancing the solubility, stability, skin penetration, and functional antioxidant activity of pterostilbene, supporting their application in advanced topical skin treatment formulations.

## CONCLUSION

This study introduces a novel nanostructured lipid carrier (NLC) system to overcome the inherent formulation limitations of pterostilbene for topical delivery. Encapsulation into NLCs markedly improved aqueous solubility, particle size uniformity, skin penetration, and antioxidant activity compared with free pterostilbene. The novelty of this work lies in the integrated demonstration that physicochemical enhancement via NLC formulation directly translates into improved biological performance and skin delivery. These findings position pterostilbene-loaded NLCs as a promising platform for topical cosmetic and dermatological applications, particularly in anti-aging and oxidative stress-related skin protection. Future studies involving in vivo evaluation and clinical validation are warranted to support their translational and commercial potential.

## AUTHOR CONTRIBUTIONS

**EL**: Conceptualization, Investigation, Data curation, Formal analysis, Validation, Writing – Original draft.
**FLY**: Methodology, Supervision, Project administration, Writing – Review & Editing, Funding acquisition.

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## COMPETING INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## ADDITIONAL INFORMATION

None.

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