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Nanoformulation of Nanostructured Lipid Carriers Encapsulating and Cytotoxicity Activity of Leum Pua (*Oryza sativa* L. variety Leum Pua) Khao–Mak Glutinous Rice Extract

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Abstract

This study aimed to develop nanostructured lipid carriers (NLCs) encapsulating Leum Pua glutinous rice extract and to evaluate their physicochemical stability and cytotoxicity. NLCs were prepared using the hot high–speed homogenization method with rice extracts obtained through oil and aqueous extraction assisted by digital ultrasonication. Montanov 82 and medium-chain triglyceride (MCT) oil served as the solid and liquid lipids, respectively, while Span 80 and Tween 20 (1:1) functioned as emulsifiers. Formulations prepared with oil-extracted rice ferment yielded opaque, pale violet dispersions that remained physically stable for at least 12 days, whereas those incorporating aqueous-extracted ferment showed sedimentation by day 4. The optimal formulation, containing 20% oil-extracted rice ferment without MCT oil, exhibited mean particle sizes of 129.6±2.16 nm and 144.5±1.46 nm, zeta potentials of −38.05±1.86 mV and −30.26 ± 0.98 mV, and polydispersity index (PDI) values of 0.193 ± 0.017 and 0.182±0.009 on storage days 8 and 12, respectively. Resazurin reduction assays confirmed >90% viability of human dermal fibroblasts at nanoparticle concentrations of 6.25–200 µg/mL over 24, 48, and 72 h, indicating no significant cytotoxicity. Overall, the findings demonstrate that oil-extracted rice ferment can serve as an effective substitute for traditional liquid lipids in NLC formulations, yielding stable and biocompatible nanoparticles with strong potential for cosmetic applications.

Introduction

Nanotechnology is an innovative area of science and technology that studies very small particles at the nanoscale level (1–100 nm) and manipulates them to produce objects or substances with new functions or special characteristics. This technology has found many applications in medicine as an effective drug delivery system. Nanoparticles are taken up by cells more efficiently than larger macromolecules and, therefore,

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can be used as effective transport systems and enable controlled release of therapeutic agents to their target locations (Patra et al., 2018). For cosmetic applications, the integration of nanoparticles into cosmetic formulations does not change the fundamental properties of cosmeceuticals but enhances the delivery and bioavailability of active ingredients. Additionally, nanoscale carriers contribute to improved skin penetration, leading to better absorption and prolonged retention of cosmetic agents, including enhanced appearance, coverage, and adherence to the skin (Gupta et al., 2022).

Lipid nanoparticles (LNPs) are nano-delivery systems made of lipids that have been developed over the past two decades as alternative carriers to traditional systems such as liposomes and polymeric nanoparticles. The structure and composition of LNPs vary depending on the types of lipids and methods used for synthesis. The first generation, solid lipid nanoparticles (SLNs), are typically composed of lipids that are solid at room temperature. Nanostructured lipid carriers (NLCs) were developed as a second-generation system to overcome problems associated with lipid crystallinity and polymorphism. In NLCs, a mixture of solid lipids and liquid lipids (oils) stabilized by surfactants forms a nanostructured, biocompatible matrix with an imperfect crystal lattice (Alfutaimani et al., 2024; Khan et al., 2022). The formulation and preparation methods of NLCs make them suitable for pharmaceutical, cosmetic, and other relevant fields. NLCs have gained considerable traction in dermatology and skincare, where they are well-suited for topical and transdermal delivery due to their ability to merge with the skin's lipid barrier and enhance the penetration of active compounds. Moreover, NLCs form an occlusive lipid film on the skin that reduces transepidermal water loss and increases hydration and skin elasticity. In cosmetics, NLCs have been heralded as innovative nanocarriers for skin care ingredients, as encapsulation within NLCs improves the stability of active ingredients and enhances their penetration into the epidermis (Chauhan et al., 2020).

In Thailand, Leum Pua is an upland cultivar of native dark purple glutinous rice, distinguished by its black pericarp. Scientific research indicates that Leum Pua rice contains higher levels of bioactive compounds, such as, total phenolics, flavonoids, and anthocyanins, compared with other colored rice varieties (Pornputtapitak et al., 2018). It is traditionally used in the preparation of Khao Mak, a sweet fermented rice dessert. While white glutinous rice varieties tend to produce higher alcohol content and total soluble solids during fermentation, Leum Pua offers superior antioxidant properties (Wongsa et al., 2018). Furthermore, Leum Pua Khao-Mak glutinous rice extract has been explored as a potential ingredient in the cosmetic industry due to its high content of bioactive compounds, such as phenolic acids and flavonoids, which possess antioxidant and anti-inflammatory properties (Wattanuruk et al., 2020a). Additionally, Leum Pua rice bran is a rich source of γ-oryzanol, a compound known for its antioxidant and cholesterol-lowering effects. Studies have explored the extraction of γ-oryzanol from Leum Pua bran and its incorporation into nanostructured lipid carriers for topical delivery, highlighting its potential application in cosmetic formulations (Pornputtapitak et al., 2018). NLCs are being developed primarily for anti-aging cosmetic applications, which are strongly associated with oxidative stress and inflammation–related skin damage. The developed NLCs are intended not only as a finished topical product but also as a versatile delivery system for the further incorporation of other active ingredients, thereby expanding their potential application in multifunctional cosmetic formulations. In this study, two different extraction methods-oil extraction and aqueous extraction–were specifically employed to evaluate their impact on the quality and performance of the resulting nanostructured lipid carriers. The selection of extraction method is critical, as solvent polarity and extraction conditions have a profound effect on the profile and concentration of bioactive compounds obtained from plant–based materials (Wijngaard & Brunton, 2009). Oil extraction tends to enrich lipophilic components such as γ-oryzanol, tocopherols, and phytosterols, while aqueous extraction predominantly yields hydrophilic compounds like phenolics and anthocyanins (Walter & Marchesan, 2011). Since these differing phytochemicals exhibit distinct physicochemical behaviors and biological activities, it is crucial to determine which extraction method produces an extract most compatible with lipid-based nanocarrier systems.

The development of Leum Pua Khao–Mak glutinous rice extract–loaded NLCs for topical delivery could add value to native Thai glutinous rice varieties. In this study, extracts were prepared using different oil extraction methods, and the compositions of each formulation were analyzed. The prepared NLC formulations were characterized using a hot high–speed homogenization method. The optimal formulation was then selected for cytotoxicity testing using the resazurin reduction assay.

Materials and methods

1. Preparation of Leum Phua Khao-Mak glutinous rice extract

The glutinous rice variety Leum Pua was purchased from Tak Province, Thailand, and was fermented according to the method described by Wattanuruk et al. (2020a). The fermented rice was dried in an oven at 60°C for 24 h. Subsequently, the dried rice samples were ground into powder and extracted using oil extraction and aqueous extraction methods (Perrier et al., 2017). The treatment variations, considered as independent variables, included the ratio of rice sample, deionized (DI) water, medium-chain triglycerides (MCT) oil (60% C8 and 40% C10; Neobee® M-5, Stepan Company, USA), and ethoxydiglycol (Table 1). For oil extraction, the rice powder was mixed with MCT oil and ethoxydiglycol until the mixture became completely homogeneous, and then subjected to green extraction using a digital ultrasonic cleaner at 37 kHz, 80°C for 30 min. After extraction, the sample was filtered through Whatman No. 1 filter paper. For aqueous extraction, the rice powder was blended with DI water and extracted under the same green extraction conditions (37 kHz, 80°C, 30 min). The resulting extract solution was centrifuged at 4,000 rpm for 10 min and subsequently filtered using Whatman No. 1 filter paper

Table 1 Ratios used for extraction methods

Extraction method	Rice sample (g)	DI water (g)	MCT oil (g)	Ethoxydiglycol (g)
Oil extraction (method 1)	20	-	50	50
Oil extraction (method 2)	40	-	50	50
Aqueous extraction	20	100	-	-

2. Development of nanostructured lipid carriers (NLCs)

Rice extract-loaded NLCs were prepared using the hot high–speed homogenization method, as described by Jiamphun and Chaiyana (2023). The oil phase, consisting of rice extract oil, sorbitan monooleate (Span 80), cetearyl alcohol and coco-glucoside (Montanov 82; SEPPIC, France), and MCT oil, as illustrated in Table 2, was mixed on a stirring hot plate at 200 rpm and 80°C until a completely homogeneous mixture was obtained. The aqueous phase, consisting of rice extract aqueous solution, polysorbate 20 (Tween 20), poloxamer 188, glycerin, chlorphenesin/phenoxyethanol (Microcare PHC eq.), and water, as shown in Table 2, was similarly mixed on a stirring hot plate at 200 rpm and 80°C until fully homogeneous. The final volume of all ten formulations was adjusted to 100.1 mL. Subsequently, the aqueous phase was added to the oil phase and stirred at 200 rpm and 80°C for 10 min. The resulting mixture was then subjected to high–speed homogenization at 10,000 rpm for 10 min to obtain a uniform dispersion and achieve the desired nanoparticle size. The physical appearance of the samples was evaluated over 12 days of storage at ambient temperature to assess stability. The size of the rice extract-loaded NLCs was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS (Malvern, Worcestershire, UK). Zeta potential and polydispersity index (PDI) values were also determined to evaluate the uniformity of the particle size distribution. Immediately after production, NLCs typically exhibited mean particle sizes in the range of 100–300 nm, with a narrow size distribution (low PDI) and high negative zeta potentials, provided that the formulation and processing parameters were optimized. NLCs with favorable characteristics, including small droplet sizes, low PDI values, and appropriate zeta potentials, were identified. All measurements, except for the evaluation of physical appearance, were performed in triplicate, with the results expressed as mean ± standard deviation (Vieira et al., 2020). Statistical comparisons of the stability parameters of formulation 10 with other formulations, as well as the effects of storage time were analyzed using one-way analysis of variance (ANOVA).

Table 2 Composition of rice extract -loaded nanostructured lipid carrier (NLC) formulations

Part	Ingredients	formular g (W/W)									
		1	2	3	4	5	6	7	8	9	10
Oil	1. Rice extract oil extraction	2	5	10	15	20	2	5	10	15	20
	2. Span 80	3	3	3	3	3	3	3	3	3	3
	3. Montanov 82	2	2	2	2	2	2	2	2	2	2
	4. MCT oil	18	15	10	5	-	18	15	10	5	-
Aq	1. Tween 20	3	3	3	3	3	3	3	3	3	3
	2. Poloxasmer 188	2	2	2	2	2	2	2	2	2	2
	3. Glycerine	2	2	2	2	2	2	2	2	2	2
	4. Chlorphenesin/ phenoxyethanol (microcare PHC eq.)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
	5. Rice extract aqueous extraction	46.4	46.4	46.4	46.4	46.4	-	-	-	-	-
	6. Water	21.1	21.1	21.1	21.1	21.1	67.5	67.5	67.5	67.5	67.5

Remark: Oil extraction (method 1) was used in formulations 1-5.
Oil extraction (method 2) was used in formulations 6-10.

3. Sample preparation for cytotoxic assay

Rice extract–loaded NLC samples were prepared in dimethyl sulfoxide (DMSO) and further diluted in culture medium to obtain final concentrations of 6.25, 12.5, 25,

50, 100, and 200 µg/mL, the final concentration of DMSO in all samples was maintained at 0.4% v/v. A vehicle control (medium containing 0.4% DMSO without NLC) was included to account for any potential solvent effects. All working solutions were freshly prepared and sterile-filtered through a 0.22 µm syringe filter prior to use. A 100% viability control (cells treated with 0.4% DMSO only) was used as a reference for cell viability calculation.

4. Cell culture

BJ human dermal fibroblasts (ATCC, USA) were maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% penicillin–streptomycin (Gibco, USA). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Subculturing was performed twice weekly using 0.25% trypsin–EDTA (Gibco, USA). Only cells with greater than 90% viability, determined by 0.4% trypan blue exclusion (Gibco, USA) at a density of 2×10^5 cells/mL, were used in each experiment (Buranrat et al., 2008).

5. Cell viability assay

The cytotoxic effects of the samples on BJ fibroblast cells were evaluated using the resazurin reduction assay. Viable cells with active metabolism reduce resazurin, a blue dye, to resorufin, a pink-colored product. BJ fibroblast cells were treated with various concentrations of the samples for 24, 48, or 72 h in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 h before treatment. After treatment, cells were incubated with 50 µg/mL resazurin solution (Sigma-Aldrich, USA) at 37°C for 4 h. Absorbance was measured at 560 nm and 600 nm using a microplate reader (VICTOR Nivo™, PerkinElmer, U.S.) Resazurin exhibits a maximum absorption at 600 nm, while resorufin absorbs maximally at 560 nm. The extent of colorimetric change was directly proportional to the number of viable cells in the sample. Cell viability (%) was calculated by comparing the absorbance values of treated samples to those of the untreated control, using the following formula (Borra et al., 2009):

Cell viability (%) = [(OD560 – OD600) sample / (OD560 – OD600) control] x 100

The viability of fibroblasts treated with formulation A was expressed as a percentage relative to the non-treated control. All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD) from three independent experiments (N = 3).

Results and discussion

1. Oil extraction and aqueous extraction of Leum Pua Khao-Mak glutinous rice

In this study, Leum Pua Khao-Mak glutinous rice was extracted using both oil extraction and aqueous extraction methods, followed by green extraction employing a digital ultrasonic cleaner. Increasing ultrasound power and exposure time disrupts the cell wall through the collision of cavitation bubbles, which generate microjets and shockwaves that physically disrupt plant or cell structures, thereby improving the release of intracellular compounds, including oils and bioactives (Gaikwad et al., 2025). Ultrasonic-assisted extraction (UAE) offers several advantages, including higher extraction yield, reduced solvent consumption, shorter processing time, and enhanced preservation of heat-sensitive compounds due to the use of mild operating temperatures (Marhamati et al., 2020). In the context of preparing nanostructured lipid carriers (NLCs), the oil extracted via UAE can be utilized in the pre-emulsion process. The improved extraction efficiency and preservation of functional compounds make UAE–extracted oils suitable for formulating stable and effective NLCs. The pre-emulsion process ensures that the active substances are effectively integrated into the nanostructure, facilitating optimal compatibility and enhancing the interactions between the active compounds and the nanoparticle system. Vigorous mixing promotes a uniform dispersion of particles, thereby increasing the surface area available for potential interactions. The optimum concentrations of rice sample–20 g for Method 1 and 40 g for Method 2–were added to MCT oil and ethoxydiglycol for oil extraction (Fig. 1a and 1b). MCT oil served both as an effective extraction solvent and as a liquid lipid component within the NLC matrix. The addition of ethoxydiglycol not only enhanced the solubilization of certain semi-polar constituents during extraction but may also contribute to improved skin penetration if the NLCs are intended for topical delivery applications. The combination of MCT oil and ethoxydiglycol under ultrasonic conditions significantly enhanced both the yield and quality of the bioactive extracts (Taha et al., 2018). Subsequently, the rice sample was extracted with deionized (DI) water for the aqueous extraction method (Fig. 1c).

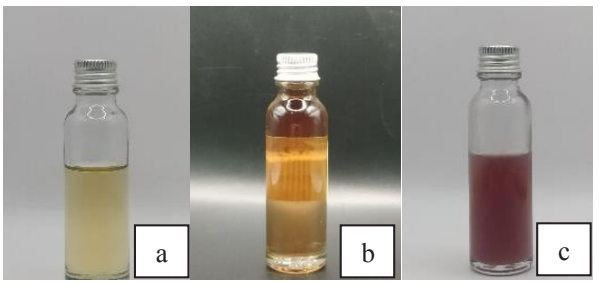

Fig. 1 Rice samples extracted using oil extraction (method 1) (a), (method 2) (b) and aqueous extraction (c)

2. Nanostructured lipid carrier (NLC) formulations encapsulating Leum Pua glutinous rice extracts

Based on the experimental results of developing nanostructured lipid carrier (NLC) formulations encapsulating Leum Pua glutinous rice extract, Montanov 82 was used as a solid lipid, while MCT oil was used as a liquid lipid. The ratios of liquid lipid were explored as determinants in the production of NLCs. Span 80 (a hydrophobic surfactant) and Tween 20 (a hydrophilic surfactant) were used as emulsifiers at a 1:1 ratio. The external appearance of the nanoparticle aqueous solutions and NLCs prepared via oil extraction (method 2) in formulations 6–10 demonstrated that all NLCs were opaque liquids with a pale violet color, resembling that of the NLC aqueous solutions (Table 3). Furthermore, NLCs containing the highest concentrate of rice extract oil exhibited the same coloration. All oil–extracted NLC formulations remained physically stable after three months of storage at ambient temperature (data not shown). In contrast, NLCs prepared using aqueous-extracted rice (method 1; formulations 1–5) showed visible sedimentation starting from day 4 of storage (Table 3), indicative of nanoparticle aggregation and instability. Moreover, phase separation was observed in these aqueous-extract-loaded formulations, highlighting their inferior stability compared to those prepared using rice oil extract. These findings clearly demonstrate the impact of extraction method on NLC performance.

The optimum NLCs exhibited small particle sizes within the range of 100–300 nm, zeta potentials between –20 and –40 mV, and polydispersity index (PDI) between 0.1814±0.00805 and 0.4299±0.02506 as shown in Tables 4 and 5, indicating a uniform size distribution. A high zeta potential, associated with increased surface charge, suggests strong repulsive forces between particles, thereby preventing aggregation. Notably, NLC aqueous solutions in formulations 9 and 10 demonstrated low polydispersity index (PDI) values, indicating a narrow particle size distribution and high uniformity of the nanostructured lipid carriers. Formulation 10, which incorporated a higher concentration (20%) of glutinous rice extract compared to formulation 9 (15%), maintained particle size uniformity, as evidenced by its low PDI. Experimental results further revealed that in formulation 10, no MCT oil was added, indicating that the rice extract oil successfully substituted for the liquid lipid in nanoparticle preparation. This not only simplified the formulation but also demonstrated that the rice extract oil was sufficiently functional as both an active and structural lipid phase. The absence of phase separation or significant particle growth in formulation 10 over the 12-day storage period confirmed its excellent physical stability, even with a higher load of active extract (20%). The ability of formulation 10 to maintain uniform nanoparticle distribution and colloidal stability without the support of added synthetic lipid components reflects its superior formulation efficiency and biocompatibility. This characteristic enhances its suitability for cosmetic applications, where the incorporation of natural actives and the use of minimal excipients are preferred. To support the evaluation of long-term physical stability, the particle size, zeta potential, and PDI of formulation 10 were measured and compared at three time points: day 0 (immediately after preparation), day 8, and day 12. On day 0, the average particle size of formulation 10 was 127.4±1.855 nm, with a zeta potential of –36.89±1.452 mV and a PDI of 0.1786± 0.00893. These values remained stable on days 8 and 12, with slight variations: 129.6±2.163 nm and 144.5 ±1.461 nm for particle size, –38.05±1.861 mV and –30.26±0.9792 mV for zeta potential, and PDI values of 0.1928±0.01745 and 0.1824±0.00900, respectively.

This comparison confirms the physical stability of the NLC system, with minimal changes in particle characteristics over time. The particle size range (100–300 nm) and zeta potential values (−20 to−40 mV) observed in this study are consistent with previous reports on NLC systems incorporating botanical extracts. For example, Jiamphun and Chaiyana (2023) reported particle sizes of 130–150 nm and zeta potentials around −35 mV in glutinous rice husk extract-loaded NLCs, indicating similar colloidal behavior and surface charge distribution. A distinguishing feature of many recent NLC formulations is the use of natural oils (obtained via extraction from plants or other sources) as the liquid lipid component. These oils serve dual roles: as structural components of the lipid matrix and, in many cases, as

therapeutically active ingredients themselves (Javed et al., 2024). By integrating extracted oils into the lipid matrix of NLCs, it is possible to combine the intrinsic bioactivity of natural oils with the carrier benefits of lipid nanoparticles. This suggests that formulation 10 effectively maintained nanoparticle stability and uniformity despite the higher active ingredient load. Consequently, formulation 10 is deemed more suitable for cosmetic applications, offering enhanced delivery of the glutinous rice extract while preserving desirable physicochemical properties of the NLC system. In contrast, NLCs prepared by oil extraction method 1 (formulations 1–5) exhibited sedimentation from day 4 of storage, suggesting aggregation of nanoparticles and the formation of larger particles, which are indicative of instability (Table 3). Additionally, the incorporation of aqueous-extracted rice extract in all formulations contributed to nanoparticle destabilization, leading to phase separation within the aqueous solutions. Since the aqueous extract was not suitable for the NLC formulation due to physical instability, anthocyanins-primarily water-soluble pigments-are unlikely to be present in the final product. Instead, the lipid extract, used successfully in stable formulations, likely retains other bioactive constituents such as γ-oryzanol, tocopherols, and phytosterols, which are known to be lipophilic and exhibit antioxidant, anti-inflammatory, and skin-protective activities. Previous studies have identified γ-oryzanol as a major active compound in rice bran oil, contributing to UV protection and skin barrier enhancement (Saewan & Jimtaisong, 2013; Pornputtapitak et al., 2018). Thus, the presence of such lipophilic compounds in the oil-extracted Leum Pua rice may support the proposed cosmetic applications of the developed NLCs. The sedimentation and phase separation observed in aqueous extract-loaded formulations align with findings by Chauhan et al. (2020), who reported instability in NLCs when the hydrophilic load exceeded lipid matrix compatibility. In contrast, formulations prepared with oil-based extracts maintained structural integrity over storage, similar to the stability trends reported by Javed et al. (2024), where oil-based actives integrated better with the lipid core and improved colloidal uniformity. This finding highlights the importance of formulation optimization and the necessity of considering the compatibility between bioactive compounds and NLC components to achieve optimal stability. By selecting appropriate components and adjusting their ratios, it is possible to enhance stability, protect bioactive compounds from degradation, and maximize the potential benefits of NLCs in various applications. Future studies should perform comprehensive phytochemical profiling of the lipid extract using advanced analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), or liquid chromatography–tandem mass spectrometry (LC–MS/MS), to quantitatively determine key bioactive constituents, including γoryzanol, tocopherols, and phytosterols. The resulting quantitative data will support claims pertaining to antioxidant, antiinflammatory, and UVprotective properties, and will also underpin the development of rigorous quality control protocols and standardization strategies for cosmetic applications. While the present study focused on particle size, zeta potential, and polydispersity index to assess the physical stability of the developed NLCs, the pH of each formulation is another critical factor that warrants further evaluation. The pH of nanocarrier systems can influence the electrostatic interactions between particles, the ionization state of encapsulated bioactives, and their compatibility with the skin. Therefore, future work should include systematic measurement of pH values across all formulations. Monitoring pH over storage time can also provide valuable insights into chemical stability and potential degradation pathways, particularly for formulations containing plant-derived actives known to be pH-sensitive. For future studies, reference to the International Council for Harmonisation (ICH) Q1A(R2) stability testing guidelines or relevant cosmetic regulatory frameworks is recommended to ensure standardized evaluation under defined conditions of temperature, humidity, and storage duration. This would facilitate regulatory acceptance, enhance reproducibility, and provide a scientific basis for shelf-life determination.

3. Cytotoxic effect of rice extract-loaded NLCs on human dermal fibroblast cells

The cytotoxicity assessment in this study was conducted using formulation 10, which was identified as the optimal NLC formulation based on its superior physicochemical stability and compatibility. The viability of cell cultures is typically assessed based on the metabolic capability of cells to convert specific chemicals into colored dyes, thereby indicating cell viability. The resazurin assay, one of the metabolic assays, is an extremely simple, rapid, sensitive, reliable, non-toxic, and inexpensive method for evaluating cell viability. Resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide) is taken up by cells, where the non-fluorescent blue dye

Table 3 Characteristics of nanoparticles prepared by different formulations and storage for 12 day

Day	Formulation									
	1	2	3	4	5	6	7	8	9	10
1										
4										
8										
9										
12										

Table 4 Particle size, zeta potential, and PDI of nanoparticles measured after 8 days of storage

Formulation	Average diameter (nm)	Zeta potential (mV)	Polydispersity index (PDI)
6	300.3±9.651	-40.13±1.374	0.4299±0.02506
7	226.5±4.469	-38.67±0.432	0.3551±0.01869
8	217.5±2.231	-38.52±1.287	0.3695±0.01891
9	142.6±3.321	-38.04±1.357	0.1893±0.01803
10	129.6±2.163	-38.05±1.861	0.1928±0.01745

Table 5 Particle size, zeta potential, and PDI of nanoparticles measured after 12 days of storage

Formulation	Average diameter (nm)	Zeta potential (mV)	Polydispersity index (PDI)
6	208.4±5.323	-40.34±0.7284	0.2981±0.01258
7	195.1±2.213	-34.45±1.1521	0.2499±0.00648
8	180.7±4.783	-29.26±0.8611	0.2512±0.00925
9	132.5±0.799	-29.44±0.1837	0.1814±0.00805
10	144.5±1.461	-30.26±0.9792	0.1824±0.00900

is reduced to the pink-colored fluorescent product, resorufin. The measurement of resorufin fluorescence provides a reliable indicator of cellular metabolic activity (O'Brien et al., 2000). This study demonstrated that the resazurin reduction assay serves as a potent tool for screening the in vitro cytotoxicity of rice extract-loaded NLC samples in human dermal fibroblast cells. The optimal conditions for performing the resazurin assay, including sample concentration and incubation time, were established to accurately assess the metabolic rates of the tested cell lines. Table 6 presents the range of sample concentrations (6.25–200 µg/mL) and incubation times for resazurin reduction (24, 48, and 72 h). The results of the resazurin assay indicated that all six sample concentrations did not exhibit cytotoxic effects after 24, 48, and 72 h of incubation, maintaining cell viability above 90%. Our previous research showed that crude extracts from Leum Pua Khao–Mak at concentrations between 0.0001 and 1 mg/mL were non-toxic to human dermal fibroblast cells (Wattanuruk et al., 2020b). The high cell viability (>90%) observed across all tested concentrations corroborates findings from previous studies involving rice-derived phytochemicals encapsulated in lipid nanoparticles. Praditya et al. (2023) and Wattanuruk et al. (2020b) both reported that rice bran or fermented rice extracts enhanced fibroblast viability, likely due to the antioxidant and cytoprotective actions of γ-oryzanol and phenolic acids. The unexpected increase in cell viability at 200 µg/mL compared to 6.25 µg/mL may be explained by a biphasic (hormetic) effect commonly observed with natural extracts and antioxidant compounds. For example, rice bran extract has previously shown cytoprotective and antioxidative effects in cardiomyocytes, suggesting a similar protective mechanism in fibroblasts (Miguel et al., 2017). Additionally, studies on black rice bran extracts have demonstrated significantly increased viability of NIH3T3 and HaCaT cells at high concentrations (>500 µg/mL), corroborating our findings (Praditya et al., 2023). Thus, the observed higher viability at 200 µg/mL may result from a synergistic combination of hormetic response and enhanced cellular uptake via the NLC system, enabling more effective protection by bioactive compounds

Table 6 Cell viability at different incubation times

Sample concentration (µg/ml)	Average of cell viability (% of untreated control)		
	24 h	48 h	72 h
0.4% DMSO	100.0±0.00	100.0±0.00	100.0±0.00
200	97.21±8.15	106.86±2.97	100.82±3.84
100	90.43±4.40	98.78±8.90	101.58±2.78
50	90.61±1.18	110.06±7.23	102.85±7.88
25	90.23±0.50	113.95±4.62	99.36±5.18
12.5	89.49±3.11	110.44±3.42	98.17±6.64
6.25	91.27±3.89	105.81±7.48	96.87±4.69

Conclusion

The present study demonstrated that nanostructured lipid carriers (NLCs) formulated with oil-extracted Leum Pua glutinous rice ferment exhibited superior physicochemical stability compared to those incorporating aqueous–extracted ferment. Formulations containing solely oil–extracted rice ferment (method 2) maintained a single–phase, homogeneous dispersion over a 12-day storage period at room temperature, whereas sedimentation and phase separation were observed in oil extraction (method 1) formulations as early as day 4. The optimal formulation, which incorporated 20% oil-extracted rice ferment without additional MCT oil, achieved small particle sizes, narrow size distributions, and highly negative zeta potentials, indicative of stable colloidal systems. Moreover, cytotoxicity assays confirmed the biocompatibility of the developed NLCs, with human dermal fibroblast viability exceeding 90% across all tested concentrations and time points. Importantly, the 12–day storage study revealed that NLCs prepared using oil extraction method 2 exhibited superior long-term stability, with no signs of aggregation or phase separation throughout the observation period. In contrast, NLCs produced using oil extraction method 1 demonstrated visible sedimentation and physical instability from day 4 onward. These findings highlight the potential of utilizing oil-extracted Leum Pua glutinous rice ferment as a dual–function component-both as a liquid lipid and an active ingredient-in the development of stable and safe NLCs for cosmetic applications. Further studies should explore the long–term stability of the NLCs under various environmental conditions (e.g., temperature, humidity, light exposure) and investigate their performance in delivering additional active cosmetic ingredients, particularly those targeting anti–aging or skin–brightening effects. Moreover, the oil extract should be further characterized to identify its chemical composition. The identified compounds could serve as markers for quality control of both the extract and the final product. In addition, reviewing the biological activities of these compounds from the literature would help support and confirm the targeted cosmetic effects of the developed formulation. For future research,

extended stability studies-covering periods of 1 to 6 months under controlled temperature and humidity conditions-are recommended in alignment with standard regulatory guidelines. Such studies would provide more comprehensive data on particle size evolution, zeta potential drift, PDI variation, and possible phase separation, thereby supporting the practical application and commercial viability of the formulation.

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