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Formulation and Antioxidant Evaluation of Anti-Aging Moisturizer Containing *Peperomia pellucida* Extract Loaded Nanostructured Lipid Carrier

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ABSTRACT

Peperomia pellucida, commonly known as Chinese betel, contains antioxidants and anti-inflammatory compounds, which are potential candidates for active ingredients in anti-aging topical moisturizer formulations. However, its low lipid solubility requires a modified delivery system, such as nanostructured lipid carriers (NLC). This study aimed to determine the optimum lipid concentration and lipid phase ratio for NLC formulation, evaluate the anti-aging potential of *Peperomia pellucida* extract-loaded NLC, and assess the characteristics of the NLC-based moisturizer. NLC was prepared using the high shear homogenization method and then characterized for its physical properties. Antioxidant activity was evaluated using the DPPH method, while the stability of the moisturizer was assessed through a six-cycle storage cycling test. NLC F1, containing 5% total lipid with a solid-to-liquid lipid ratio of 3:2, had the smallest particle size among all formulations and was selected as the optimal formula. The antioxidant test of NLC F1 revealed a spheroidal morphology, confirmed by TEM, and an IC50 value of 113.45 ppm, indicating moderate antioxidant activity. When incorporated into a moisturizer, the formulation maintained stable organoleptic properties, homogeneity, pH, spreadability, and adhesiveness over six test cycles, despite a decrease in viscosity. The optimized lipid composition produced a stable NLC-based moisturizer loaded with *Peperomia pellucida* extract, showing moderate antioxidant activity and favorable physicochemical characteristics, supporting its potential as an anti-aging cosmeceutical product.

***Keywords*:** Anti-aging; Chinese betel; Nanostructured lipid carrier; Moisturizer; *Peperomia pellucida*

INTRODUCTION

The COVID-19 pandemic has significantly shifted global awareness toward the use of cosmetic products. Cosmetics are categorized into decorative (makeup) and skincare products. During the pandemic, public interest in skincare products increased, while interest in makeup declined.[1] Active ingredients with therapeutic effects are frequently incorporated into skincare, resulting in cosmeceutical formulations.[2] According to Dr. Kligman, cosmeceuticals are "topical preparations sold as cosmetics but containing biologically active ingredients purporting to have pharmaceutical therapeutic benefits." The cosmetics industry often uses this term to highlight

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the specific mechanisms of active compounds in modern skincare products.[3]

Indonesia's tropical climate results in constant exposure of the skin to sunlight, making ultraviolet (UV) rays a major external factor in skin aging. Pollution and smoking further accelerate degenerative skin processes, leading to wrinkles, loss of skin elasticity, and the appearance of age spots beginning in the twenties.[4] According to the ZAP Beauty Index 2023, Indonesian women across generations are increasingly aware of anti-aging benefits. Data show that 80.2% of Generation X, 64.0% of Generation Y, and 34.2% of Generation Z seek anti-aging benefits in their skincare products.[5] The global anti-aging cosmetics market is projected to grow at 5.99% from 2023 to 2032, with Asia-Pacific (including Indonesia) and Europe representing the largest regional markets.[6] A recent study on Chinese women aged 20–40 found that perceived age often exceeds chronological age, with deeper skin tone being the key contributor to older appearance in women in their 20s, while wrinkles, pigmentation, and mid-face roughness dominate in their 30s.[7] This emphasizes the importance of early intervention strategies in anti-aging skincare. With the rising concern about skin aging, the public is becoming more proactive in its prevention and treatment. In high-humidity climates such as Indonesia, maintaining adequate skin moisturization is essential not only for hydration but also for supporting the skin barrier function that protects against oxidative stress and environmental aggressors. Proper moisturization helps reduce transepidermal water loss, preserving skin elasticity and enhancing the efficacy of antioxidant compounds.[8] Therefore, the combination of moisturization and antioxidant activity is expected to provide synergistic anti-aging effects.

Peperomia pellucida (Chinese betel) contains antioxidant compounds (velutin, citronellol, and phytol) and anti-inflammatory compounds (elemol, β-caryophyllene, linalyl acetate, and neointermedeol) with potential as topical anti-aging moisturizers, capable of treating dry skin and maintaining hydration. Previous studies have reported that *P. pellucida* extract exhibits significant antioxidant and anti-inflammatory activities, supporting its potential for topical anti-aging applications. The 5% concentration used in this formulation was selected based on preliminary optimization studies to ensure formulation stability, homogeneity, and effective delivery of bioactive compounds without compromising skin compatibility. However, the bioactive compounds possess low lipid solubility, limiting their penetration into target tissues.[3,9]

To address this limitation, nanostructured lipid carriers (NLCs) have emerged as a delivery system that combines solid and liquid lipids to enhance dermal absorption of poorly soluble compounds. Lipids commonly used in NLCs include triglycerides, fatty acids, steroids, and waxes. Lipid selection is based on physicochemical properties at room temperature, drug solubility, compatibility between solid and liquid lipids, and toxicity profile. NLCs enhance the delivery of poorly lipid-soluble active ingredients by improving dermal penetration and maximizing efficacy. Additionally, NLCs protect active compounds within the lipid matrix, enhancing their stability.[10] The objective of this study is to develop a stable anti-aging cosmeceutical moisturizer formulation containing *Peperomia pellucida* extract using NLC technology, and to evaluate its physical characteristics and antioxidant activity.

METHODS

Extraction

Peperomia pellucida leaf powder was purchased from Balai Materia Medika, Batu, Indonesia. A total of 250 grams of powdered *Peperomia pellucida* leaves was macerated using ethanol 70% (Onemed) at a ratio of 1:5 (250 g in 1.25 L). The maceration process lasted for five days. The resulting extract was filtered and

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concentrated using a rotary evaporator (IKA RV10 Basic V) at 40°C. The extract was transferred into a porcelain dish, weighed, sealed, and stored in a refrigerator.

NLC Preparation

Three formulations of NLC (Table 1) were prepared using the high shear homogenization method. The aqueous and surfactant phases were heated to 80–85°C, and the surfactant was dissolved into phosphate buffer (Merck, pH 7.4). The lipid phase was melted, and Span 20 (Labotiq) was preheated to the same temperature. The molten lipid phase was homogenized using an Ultra-Turrax (IKA®T250) at 8000 rpm. Span 20 (Labotiq) was added during homogenization. Once the lipid phase became a uniform off-white mixture, *Peperomia pellucida* extract was incorporated. The aqueous phase was gradually added while maintaining constant stirring for 30 minutes at 80–85°C. The final mixture was cooled in a water bath (Thermo Fisher) with stirring (800 rpm) until reaching 33°C, then stored at 2–8°C.

Table 1. Preparation of the *Peperomia pellucida* NLC system

Ingredients	F1 (%)	F2 (%)	F3 (%)
Peperomia pellucida extract	5	5	5
Glyceryl monostearate	3	6	10
Oleic acid	2	4	5
Tween 80	0.5	0.5	0.5
Span 20	9.5	9.5	9.5
Phosphate buffer (pH 7.4)	ad 100	ad 100	ad 100

NLC formulations were prepared to optimize the lipid composition and particle stability of Peperomia pellucida extract. Glyceryl monostearate (CIMS) and oleic acid (Labotiq) were used as the solid and liquid lipid phases, respectively, to form a stable nanostructured lipid matrix capable of encapsulating the extract. Tween 80 (Labotiq) and Span 20 (Labotiq) acted as surfactant and co-surfactant to reduce interfacial tension, facilitating the formation of nanosized particles with uniform dispersion. Phosphate buffer (Merck, pH 7.4) served as the aqueous phase and maintained the physiological pH suitable for topical application. The concentration of 5% extract was selected based on preliminary stability and activity screening to ensure optimal encapsulation and skin compatibility. The optimized NLC was subsequently incorporated into the moisturizer formulation (Table 2).

Particle Size, Polydispersity Index (PDI), Zeta Potential (ZP), and Morphological Evaluation

Particle size, PDI, and ZP were measured using a particle size analyzer (PSA) (Shimadzu®SALD-7500). Samples were diluted 200 times (50 µL of 500 ppm stock diluted to 10 mL with distilled water (Hydrobat)). Meanwhile, morphology was observed via transmission electron microscopy (TEM) (JEM-1400Flash) at Universitas Gadjah Mada (UGM). Samples were diluted 10 times (50 µL sample + 450 µL distilled water (Hydrobat)).

Antioxidant Activity Test

Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Optimal NLC formulation as samples (F1) and standard (ascorbic acid (Merck)) were diluted with ethanol (Onemed) to 1000 ppm and serially diluted into five concentrations (40, 60, 80, 100, 120 ppm). Each sample (1 mL) was mixed with 2 mL of 1000 ppm DPPH (Sigma Aldrich) solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm using UV-Vis spectrophotometry (Shimadzu UV-1800). Percent inhibition was calculated by:

$$\% \text{ Inhibition} = \frac{A_{\text{Blanko}} - A_{\text{Sample}}}{A_{\text{Blanko}}} \times 100$$

where A_{Blanko} is the absorbance of DPPH solution with solvent (no sample) and A_{Sample} is absorbance with sample. The IC_{50} (concentration that produces 50% inhibition) was calculated from the concentration–response curve. IC_{50} was estimated by (a) linear interpolation between the concentrations flanking 50% inhibition.[11]

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Preparation of NLC-Based Moisturizer

NLC formulation (F1) (Table 2) was selected for incorporation into the final moisturizer formulation. The formulation involved heating distilled water to 70–75°C, dissolving methylparaben (CV. Nurra Gemilang) and propylparaben (CV. Nurra Gemilang), and incorporating preheated Tween 80 (Labotiq). The oil phase (glyceryl monostearate (CIMS), Span 20 (Labotiq), white petrolatum (CV. Nurra Gemilang)) was melted and homogenized using an overhead stirrer. The aqueous phase was slowly added to the oil phase and mixed for 30 minutes without further heating. The final homogeneous yellowish-white moisturizer was stored for quality evaluation.

Table 2. Moisturizer Formulation

Ingredients	Function	Concentration (%)
NLC *Peperomia pellucida*	Active compound	5
Tween 80	Emulsifier	4
Span 20	Co-Emulsifier	6
Glyceryl monostearate	Emollient	4.5
White petrolatum	Occlusive agent	6
Methylparaben	Preservative	0.4
Propylparaben	Preservative	0.4
Distilled water	Aqueous phase	ad 100

Evaluation of Moisturizer Quality

The moisturizer formulation was evaluated for organoleptic properties, homogeneity, pH, viscosity, spreadability, adhesiveness, and physical stability. The organoleptic evaluation showed a vaseline-like aroma, yellowish-white color, and semi-solid consistency, indicating acceptable sensory characteristics. The formulation appeared homogeneous, with no visible lumps or phase separation. The pH value determined using a TOA-Dkk, measured by dispersing 1 g of sample in 10 mL of distilled water (Hydrobat), was within the acceptable range of 4.5–8.0, suggesting good skin compatibility and minimal irritation potential. Viscosity, determined using a Rion viscometer, met the internal specification for semi-solid moisturizers, ensuring stable consistency and ease of application. The spreadability test, performed by placing 1 g of sample between two glass plates under a 200 g load, resulted in a spread diameter of 5–7 cm, reflecting good extensibility on the skin surface. Adhesiveness testing, conducted by pressing two glass plates with a 1 kg load, showed an adhesion time exceeding 4 seconds, indicating satisfactory adherence and prolonged moisturizing effect. Physical stability was assessed using the freeze–thaw cycling method, in which samples were alternately stored at 4 ± 2°C and 40 ± 2°C for six cycles (12 days). No changes in color, odor, phase separation, pH, or viscosity were observed after the cycling test, confirming that the formulation was physically stable and met the required quality specifications for topical use.

RESULTS AND DISCUSSION

Extraction Yield

The *Peperomia pellucida* leaf powder used in this study appeared green and was extracted using the maceration method. A total of 38.3 grams of extract was obtained from 250 grams of powdered simplicia, corresponding to a yield of 15.32%. According to the Indonesian Herbal Pharmacopoeia (2nd Edition), the minimum acceptable yield for this plant material is 13.1%.[12,13] Therefore, the obtained yield was considered ideal, confirming the efficiency of the extraction process.

Characterization of NLC

The physical characteristics of the NLC formulations were evaluated, including particle size, polydispersity index (PDI), and zeta potential (ZP), as shown in **Table 3**. All three formulations exhibited particle sizes within the acceptable nanoparticle range (10–1000 nm), with F1 displaying the smallest average particle size (208.4 nm), which is favorable for enhanced dermal penetration. Literature supports that lipid nanoparticles smaller than 300 nm can

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effectively penetrate the epidermis and reach the dermis.[14]

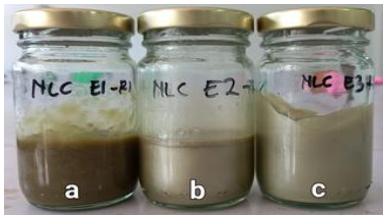

Figure 1. NLC. a) F1, b) F2, and c) F3

Table 3. Particle Size, Polydispersity Index, and Zeta Potential of NLC Formulations

Sample	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	208.4	0.254	0
F2	260.2	0.16	−10.7
F3	235.9	0.231	−26.7

The PDI values for F1, F2, and F3 indicated a narrow and homogeneous particle size distribution. The use of Tween 80 and Span 20 as surfactant-stabilizers likely contributed to emulsion stability and consistent droplet formation, as seen in the low PDI values of the formulations. Zeta potential analysis showed that F2 and F3 carried negative surface charges (−10.7 mV and −26.7 mV, respectively), within the acceptable threshold for colloidal stability. In contrast, F1 exhibited a ZP of 0.0 mV, indicating neutral surface charge and minimal electrostatic repulsion between particles. This condition may increase the likelihood of particle aggregation over time, thus potentially reducing long-term colloidal stability. Although F1 was selected as the optimal formulation based on its favorable particle size, its low zeta potential raises concerns regarding stability. This observation aligns with general NLC design principles, where ZP values exceeding ±30 mV are considered ideal for enhanced repulsive interactions and long-term dispersion stability.[10] According to the characterization results, F1 was identified as the optimal NLC formulation. To ensure a long-term profile, further studies were carried out to evaluate the antioxidant activity of the NLC F1 in biological systems and to assess the stability of the NLC F1-based moisturizer.

The morphology of the optimal NLC formulation (F1) was evaluated using transmission electron microscopy (TEM). As demonstrated in **Figure 2**, the NLC F1 particles appeared spherical in shape with uniform dispersion. This confirms the successful formation of nanostructured particles within the desired size range and supports the physical characterization results previously reported in **Table 3**. The spherical and sterically dispersed morphology observed in TEM images of NLC F1 is consistent with well-formed lipid nanoparticles, which are known to contribute to both physical stability and uniform dermal delivery.

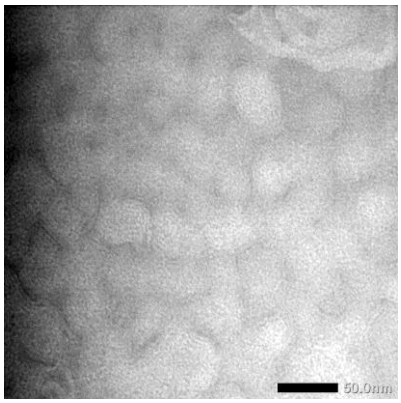

Figure 2. TEM Image of NLC F1. Magnification 80,000×, scale bar 50 nm

Antioxidant Activity of NLC

The antioxidant activity of NLC F1 was assessed using the DPPH assay and compared with ascorbic acid as a positive control. The IC_{50} value of NLC F1 was 113.45 ppm, which is categorized as moderate antioxidant activity. In contrast, ascorbic acid exhibited a significantly lower IC_{50} value of 0.97 ppm, reflecting very strong antioxidant potential. Both assays demonstrated high linearity, with R^2 values of 0.988 for NLC F1 and 0.9866 for ascorbic acid. The percentage inhibition curves for both samples are shown in Figure 3 and Figure 4.

Previous studies on unformulated *Peperomia pellucida* extract have reported lower IC_{50} values, around 80 ppm[9], suggesting stronger antioxidant activity when the extract is applied directly. The higher IC_{50} observed in this study may be

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attributed to the encapsulation process, which reduces the immediate bioavailability of active compounds due to dilution and controlled release from the lipid matrix. Additionally, some degradation of antioxidant constituents could have occurred during formulation or storage. [14,15]

Despite this, the moderate activity achieved remains relevant for cosmeceutical applications, where a sustained release of antioxidants is preferred for long-term protective effects. The use of nanostructured lipid carriers also enhances dermal penetration and protects the active agents from environmental degradation. These results indicate that both the bioactive extract and the delivery system significantly influence the overall antioxidant performance of the final product.

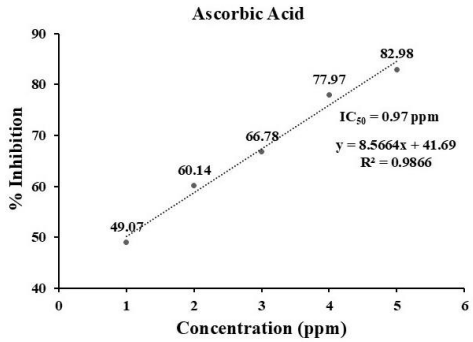

Figure 3. Antioxidant Activity of Ascorbic Acid

Evaluation of NLC-Based Moisturizer

The optimal NLC formulation (F1) was incorporated into a moisturizer base. The resulting product was a yellowish-white, semi-solid emulsion with uniform consistency (Figure 5), which remained visually stable over the physical stability test (Table 4).

The stability of the moisturizer was assessed through six cycling test rounds, evaluating organoleptic parameters, homogeneity, pH, viscosity, spreadability, and adhesiveness (Table 4; Figures 6-9). The formulation retained its yellowish-white color, semi-solid consistency, and vaseline-like aroma, fulfilling standard organoleptic criteria for cosmetic emulsions.[16]

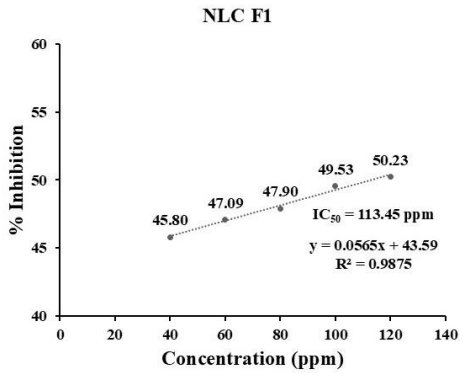

Figure 4. Antioxidant Activity of NLC F1

Figure 5. NLC-Based Moisturizer Appearance

Homogeneity remained acceptable throughout, with no observed clumping or phase separation.[17] pH values ranged between 8.04 and 8.19 (Figure 6), which, though slightly alkaline, are still within the acceptable dermal tolerance range (4.5–8.0) for cosmetic products [18]. The slight elevation could be due to surfactant and preservative effects, but did not cause significant product degradation or instability. Viscosity decreased from 3.0 dPa to 1.1 dPa over the test cycles (Figure 7). This decline is attributed to temperature fluctuations during alternating cold (4°C) and hot (40°C) storage, which disrupt molecular cohesion in semi-solid emulsions and increase atomic spacing.[19] Consequently, spreadability increased from 6.3 cm to 8.6 cm (Figure 8), as the formulation became less viscous. Though higher spreadability may improve user experience during application, it also indicates potential instability under long-term storage without further stabilization strategies. Adhesiveness remained stable across all cycles (>6 seconds), as shown in Figure 9, meeting the minimum requirement for skin retention.[20]

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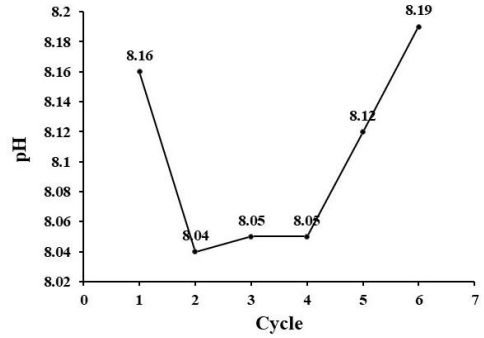

Figure 6. pH Profile of NLC-Based Moisturizer Over Six Storage Cycles

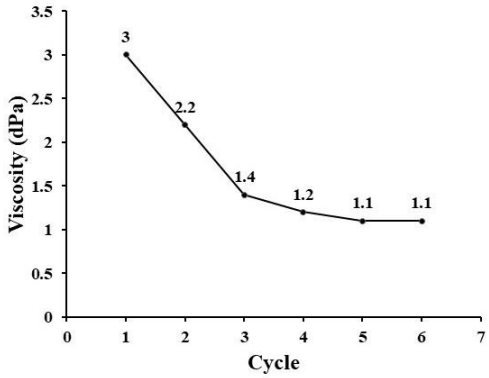

Figure 7. Viscosity Profile of NLC-Based Moisturizer Over Six Storage Cycles

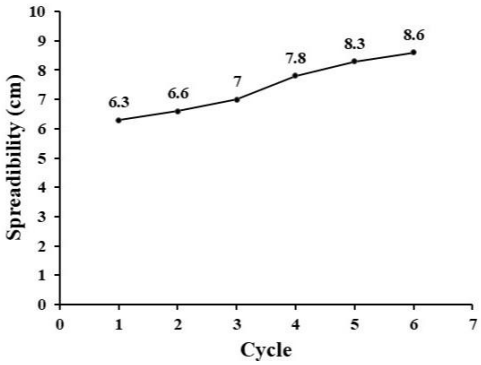

Figure 8. Spreadability Profile of NLC-Based Moisturizer Over Six Storage Cycles

The evaluation focused on the NLC-based moisturizer containing formulation F1, which was selected based on its optimal physicochemical characteristics, including the smallest particle size, narrowest distribution, and highest dispersion stability compared to F2 and F3. Smaller and narrowly distributed NLC particles exhibit higher physical stability, reduced aggregation tendency, and improved biocompatibility, which are essential for maintaining formulation integrity and enhancing drug delivery efficiency.[21]

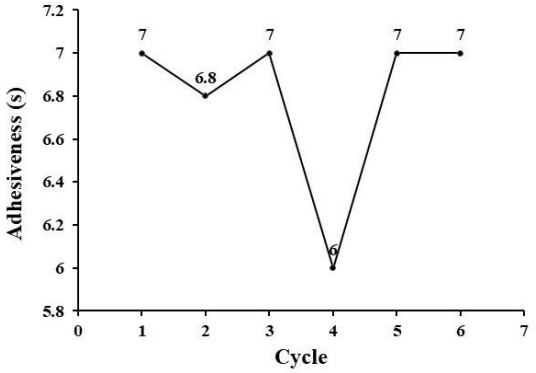

Figure 9. Adhesiveness Profile of NLC-Based Moisturizer Over Six Storage Cycles

These parameters are critical indicators of formulation performance and are directly associated with antioxidant efficiency and product consistency. Although the NLC formulations differed in the concentration of glyceryl monostearate (3-10%) and oleic acid (2-5%) in NLC, these lipids were present in very small amounts within the final moisturizer, which contained only 5% NLC in the total formulation. Consequently, their effective concentrations in the final product were below 0.5%, insufficient to significantly influence the overall viscosity, spreadability, or stability of the moisturizer.[22] The physicochemical properties of the base components particularly the emulsifiers (Tween 80, Span 20), emollient (glyceryl monostearate) and occlusive agent (white petrolatum) played a more dominant role in determining the stability and texture of the final emulsion. Therefore, any minor variations in lipid composition among NLC formulations were unlikely to produce observable differences in the stability profile of the NLC-based moisturizer. Future work will include a more detailed comparative evaluation to confirm this finding.

Overall, the NLC-based moisturizer fulfilled physical quality standards and demonstrated stability under stress conditions, including multiple storage cycles. These findings support its potential as a viable anti-aging cosmeceutical

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Table 4. Physical Stability Parameters of Moisturizer Across Six Storage Cycles

Parameter	Specification	Initial Test	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
Aroma	Vaseline-like	Vaseline-like	Vaseline-like	Vaseline-like	Vaseline-like	Vaseline-like	Vaseline-like	Vaseline-like
Color	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Yellowish white
Texture	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	4.5–8.0	8.16	8.16	8.04	8.05	8.05	8.12	8.19
Viscosity	Internal specification	3.0 dPa	3.0 dPa	2.2 dPa	1.4 dPa	1.2 dPa	1.1 dPa	1.1 dPa
Spreadability	5–7 cm	6.3 cm	6.3 cm	6.6 cm	7.0 cm	7.8 cm	8.3 cm	8.6 cm
Adhesiveness	> 4 seconds	7 s	7 s	7 s	6.8 s	7 s	7 s	7 s

formulation utilizing *Peperomia pellucida* extract. The formulation combined moderate antioxidant activity with favorable physicochemical characteristics suitable for topical application. To the best of our knowledge, this is the first report describing a stable topical moisturizer incorporating *Peperomia pellucida* extract into the NLC system for anti-aging purposes.

CONCLUSION

Based on the research findings, it can be concluded that the optimal total lipid concentration for the preparation of nanostructured lipid carriers (NLC) was 5%, with a solid-to-liquid lipid ratio of 3:2. Among the three NLC formulations developed (F1-F3), formulation F1 demonstrated the most favorable physicochemical characteristics, making it the optimal formulation for further development. The optimized NLC F1, with moderate antioxidant (IC_{50} value of 113.45 ppm) was successfully formulated into a moisturizer that remained physically stable throughout six freeze-thaw storage cycles, maintaining acceptable values for pH, viscosity, spreadability, adhesiveness, homogeneity, and organoleptic properties. Overall, these results highlight the potential application of NLC F1-based moisturizer as an anti-aging formulation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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