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Formulation and Characterization of Nanostructured Lipid Carriers Composed of Solid Lipids (Cetyl Palmitate and Stearic Acid) and Liquid Lipid (Nyamplung Oil [*Calophyllum inophyllum* L.])

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ABSTRACT

Nanostructured lipid carriers (NLCs) are lipid-based drug delivery systems made from a mix of liquid and solid lipids, stabilized by surfactants. Nyamplung (*Calophyllum inophyllum* L) oil, rich in oleic acid (48.49%), has shown in recent studies to enhance wound healing and dermatological performance when used in formulations like bigels and hydrogels. The present study aimed to formulate and characterize NLC system composed of solid lipids (cetyl palmitate and stearic acid) and a liquid lipid (nyamplung oil) for effective drug delivery. Six different NLC formulations (I-VI) were prepared by varying the concentrations of cetyl palmitate, stearic acid, and nyamplung oil. The NLC formulations were characterized by assessing their pH, viscosity, particle size, and polydispersity index (PDI). The particle size analysis revealed that the majority of formulations had particle sizes below 1000 nm, which is consistent with the nanoscale range needed for NLC systems, while the viscosity values confirmed good consistency and homogeneity. Uniform particle production and stability were suggested by the monodisperse distributions shown by the PDI values. For all evaluations, the physical properties of Formulations I–III generally satisfied the required standards. Meanwhile Formula VI's particle size exceeded 1000 nm, indicating a less stable dispersion at that lipid ratio. Formulations IV–VI also showed acceptable physicochemical stability. Notably, the use of nyamplung oil contributed additional benefits, such as antioxidant and anti-inflammatory properties, enhancing the overall therapeutic potential. The study highlights the use of locally available natural ingredients to innovate lipid-based delivery systems, offering promising applications in cosmeceutical and pharmaceutical formulations.

Keywords: Nanostructured lipid carriers, *Callophyllum inophyllum*, cetyl palmitate, stearic acid, oil, FTIR.

Introduction

The development of a carrier system based on solid lipid nanoparticles, known as the nanostructured lipid carrier (NLC) system, has some limitations. It exhibits a low rate of diffusion, which results in a long release time. Furthermore, its high-water content promotes crystallization in the system and ultimately lowers the solubility of bioactive compounds and can lead to bursts or sudden releases.[1] A lipid-based carrier system called NLC combines a matrix of liquid and solid lipids (Figure 1).[2] Drug entrapment may be possible in mixtures of liquid and solid lipids. The system will remain in a solid lipid state and enable controlled drug release by controlling the concentration of liquid lipids to be added to the formula.[3] The most often utilized solid lipids for NLC production are cetyl palmitic acid, stearic acid, glyceryl behenate, glyceryl palmitostearate, and glyceryl monostearate/monostearate.

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Caprylic acid and medium-chain triglycerides are the most often utilized liquid lipids for the creation of NLC.[4] A naturally occurring saturated fatty acid, stearic acid, is less hazardous than the synthetic group and has superior biocompatibility.[5] Additionally, stearic acid oxidizes more slowly than unsaturated lipids. To create a lipid core matrix stabilized by surfactants, the NLC system is made up of homogenized liquid and solid lipids. The properties of NLC, including particle size, zeta potential, entrapment efficiency, pH, viscosity, polydispersity index (PDI), and physicochemical stability, are significantly influenced by lipid components. The development of the lipid core matrix will occur when a solid lipid phase is added. Because stearic acid solid lipids have particle sizes between 214.7 and 237.3 nm, this study was proposed to create an NLC system with a stearic acid solid lipid phase that can reduce particle size and improve medication penetration through the skin to the greatest extent possible.[6] Cetyl palmitate solid lipids are used because they are stable at room temperature (25˚C) and have tiny particle sizes.[7]

Nyamplung (*Calophyllum inophylum* L.) is a tropical evergreen tree (Figure 2) and is a member of Calophyllaceae family. It is usually found in the coastal areas of the Southeast Asia, Pacific Islands and some regions of Africa. Nyamplung oil is a type of vegetable oil characterized by a high fatty acid content, with oleic acid comprising approximately 48.49% of its composition. In this instance, oleic acid has the benefit of not being readily oxidized by the crystallization process when the NLC system's temperature drops because it can slow down the crystallization process and influence the active ingredient's release rate.[8] Nyamplung oil is advantageous because it can treat skin conditions like psoriasis, dermatitis brought on by free radicals, and skin ageing. The body needs protection because free radicals will harm its cells.

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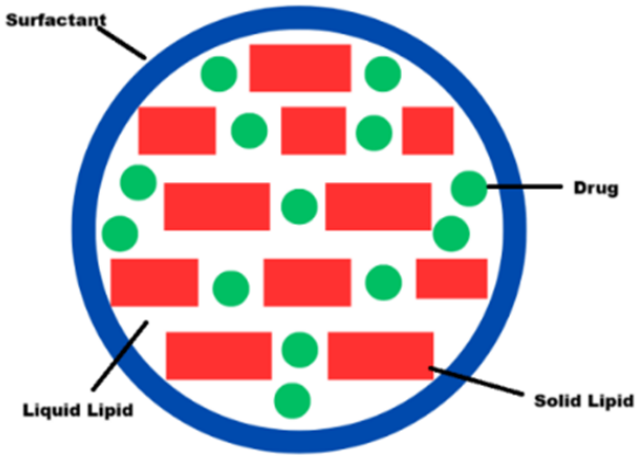

Figure 1: Nanostructured lipid carriers.[1]

Figure 2: Nyamplung (*Calophyllum inophyllum* L.)

Oleic acid, linoleic acid, palmitic acid, and stearic acid are among the fatty acids found in nyamplung oil that can both moisturize the skin and act as a drug carrier. Furthermore, nyamplung oil's liquid lipid phase can protect and hydrate the skin while acting as an antioxidant.[9] Recent evidence has demonstrated that formulations based on nyamplung oil, both in bigels and hydrogel systems, can enhance wound healing and improve dermatological performance. Moreover, the literature highlights the remarkable bioavailability and natural abundance of this oil in Indonesia, with seed oil content ranging from 65% to 75%. These characteristics make nyamplung oil a promising candidate from the perspective of local availability and sustainability.[10]

This research was conducted to determine the physical characteristics of the NLC system formulated with solid lipids (cetyl palmitate and stearic acid) and a liquid lipid (nyamplung oil). Six formulations were prepared by varying the concentrations of cetyl palmitate-nyamplung oil and stearic acid-nyamplung oil to obtain an NLC system with desirable physical characteristics, including optimal particle size, PDI, pH, and viscosity. The utilization of vegetable oils as the liquid lipid phase in the preparation of the NLC systems remains relatively limited. The novelty of this research lies in the employment of nyamplung oil as a locally sourced liquid lipid phase from Indonesia for the development of nanostructured lipid-based delivery systems. Beyond serving as the liquid lipid phase, nyamplung oil also exhibits antioxidant and anti-inflammatory activities, thereby enhancing the therapeutic potential and effectiveness of the NLC system as a drug delivery platform. Furthermore, cetyl palmitate and stearic acid were utilized as solid lipid phases to achieve balanced structural rigidity within the lipid matrix and to ensure a controlled drug release profile. Overall, this formulation strategy not only contributes to the advancement of NLC technology but also supports the innovation of natural ingredient–based cosmetic and pharmaceutical products in Indonesia.

Materials and Methods

Source of chemicals

The materials used in this study were of industrial grade purity. Nyamplung oil (100%) was obtained from Samtamanu Ltd. Other materials included cetyl palmitate (Brataco Ltd.), stearic acid (Brataco Ltd.), Tween 80 (Brataco Ltd.), propylene glycol (Brataco Ltd.), and aquadest (Multi Kimia Raya Ltd.).

Identification of nyamplung oil and its formulas

The nyamplung oil was identified with a Fourier transform infrared (FTIR) spectrophotometer (PerkinElmer Spectrum Version 10.4.00, US) to verify the purity of the oil.[11] Following the acquisition of the FTIR data, the infrared (IR) spectrum data was examined by contrasting the wave numbers in the nyamplung oil IR spectrum with the functional group absorptions in the IR spectrum sample formulas.

Preparation of nanostructured lipid carriers

The NLCs were prepared with cetyl palmitate, stearic acid, nyamplung oil, Tween 80, propylene glycol, and distilled water. The emulsification method was employed using an Ultra-Turrax T25 high-shear homogenizer (HSH). Tween 80 was heated and stirred at 800 rpm and 80°C using a magnetic stirrer, after which propylene glycol was added at a stirring speed of 1000 rpm. The cetyl palmitate, stearic acid, and nyamplung oil were then added sequentially while the stirring speed was increased to 1,100 rpm, followed by the addition of distilled water. The resulting mixture was homogenized at 24,000 rpm for 5 minutes at 80°C and subsequently allowed to cool to room temperature (25°C). The preparation of the NLC system was carried out in three replications to ensure reproducibility and reliability of the formulation process before subsequent evaluations and characterization tests.[1] Six formulas were obtained in the formulation, as shown in Table 1.

Table 1: Formulation of nanostructured lipid carriers.

Ingredient (% w/w)	Formula					
	FI	**FII**	**FIII**	**FIV**	**FV**	**FVI**
Nyamplung oil (NO)	3	2	1	3	2	1
Cetyl palmitate (CP)	3	4	5	0	0	0
Stearic acid (SA)	0	0	0	3	4	5
Tween 80	23	23	23	23	23	23
Propilenglycol	10	10	10	10	10	10
Distilled water up to	100	100	100	100	100	100

Evaluation of the nanostructured lipid carrier pH

A calibrated OHAUS-AB41PH, AQUASEARCHERTM Benchtop pH Meter was used to measure the pH of the NLC. After inserting the electrode into the preparation, the pH meter's reading was recorded.

Evaluation of the nanostructured lipid carrier viscosity

A viscosity examination was conducted with a DVNext Wells-Brookfield Cone (DVNext, US) and Plate Rheometer (DVNext, Ametex Inc., US). Rotor No. 3, which is mounted on an iron viscometer and locked counterclockwise, was utilized. A 150 ml NLC sample was placed in the cup, and the sample was ensured to be free of bubbles and spread evenly on the surface of the cup. After that, the rotor was installed in the middle of the cup containing the NLC sample, then the device was turned on. The rotor rotated, and the viscosity pointer moved to the right.

Evaluation of particle size and size distribution

Evaluation of particle size and size distribution was carried out with the HORIBA SZ-100 Particle Size Analyzer (SZ-100, Horiba Ltd., Jepang). One gram of the sample was weighed, then distilled water was added to a volume of 10 mL. If a cloudy sample was obtained, the sample was diluted 10 times. After the sample was placed inside the cuvette, the cuvette was placed inside the sample holder. The equipment was switched on, and the particle size menu was selected. Measurements were taken for the PDI and average droplet diameter. The NLC sample

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was assessed at 25˚C room temperature.

Statistical analysis
The data were analyzed using the Statistical Package for Social Sciences (SPSS; IBM Corp; version 21), and the values are expressed as means ± standard errors of the mean (SEM). Differences between groups were evaluated by analysis of variance (ANOVA) with the Bonferroni post hoc test. Statistical significance was set at $p < 0.05$.

Results and Discussion

FTIR spectral characteristics of nyamplung oil and its NLC formulas
The results of the FTIR spectrophotometric analysis (Figure 3) showed that nyamplung oil exhibited characteristic functional groups. These functional groups included C=O (carbonyl ester), C-O (ester), C-H (alkane), C-H (aliphatic) and $–CH_3$ groups. It was observed that the absorption bands corresponding to the sp^3 C–H stretching of alkanes appeared at 2926.6 cm^{-1} and 2856.5 cm^{-1}. The C=O stretching vibration of carbonyl esters was observed at 1745.94 cm^{-1}, while the C=C stretching appeared at 1624.33 cm^{-1}. Furthermore, the absorptions of the CH_2-CH_2 bending were detected at 1458.49 cm^{-1} and 1378.78 cm^{-1}. The presence of C-NO and CO bonds was also indicated by the absorption band at 1164.31 cm^{-1}. The results' interpretation showed that methyl ester compounds from unsaturated fatty acids exhibit functional group absorptions. The test results demonstrated that there was little difference between the spectrum of the used nyamplung oil and the previous FTIR analysis of the oil.[12]. The IR spectrum of the NLC system formulated with stearic acid, cetyl palmitate and nyamplung oil showed characteristic absorption peaks corresponding to the functional groups of the initial lipid components. These distinctive peaks in the NLC spectrum indicated that the lipid matrix's basic chemical structures were preserved during the production of the nanoparticles. However, slight shifts in the absorption bands and changes in intensity suggest physical interactions or molecular rearrangements between the solid and liquid lipids, confirming the successful formation of the NLC system while maintaining the original chemical integrity of the lipid constituents.

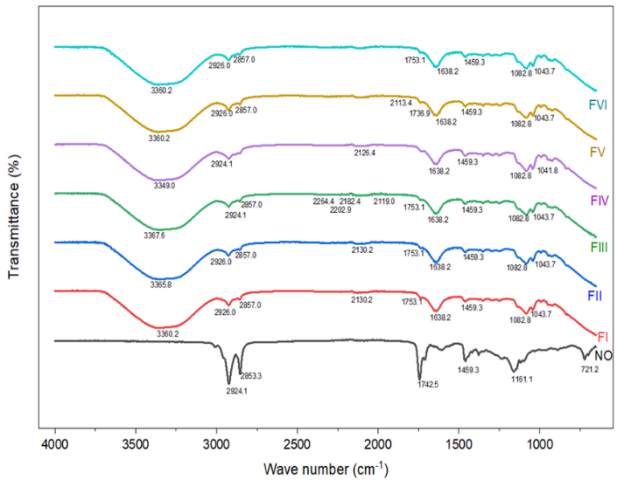

Figure 3: Profile of Fourier transform infrared (FTIR) spectrum of nyamplung oil.

Physical characteristics of the nanostructured lipid carriers
The final pH of the NLC formulas was between 4 and 6 (Table 2), which is the skin pH range. Tween 80 has a pH of 2.0.[13] Propylene glycol has a pH of 3-6.[14] Cetyl palmitate has a pH of 5.69, compared to 5.36 for nyamplung oil. The NLC system's viscosity level was ascertained by the use of viscosity measurement. Formula I (CP 3: NO 3) and Formula III (CP 5: NO 1) exhibited lower viscosities than Formula II (CP 4: NO 2), according to the results of the viscosity test conducted on Formulas I–III. The particle size obtained from Formulas I–III was less than 1000 nm. It may be inferred from this observation that the three formulas' particle sizes fall within the 10-1000 nm range of the NLC system. Based on the results (Figure 4; Table 2) of the particle size tests for the three formulas, Formula II (CP 4: NO 2) had the sample with the smallest particle size. In theory, Formula I (CP 3: NO 3) will produce a smaller particle size even though it contains less solid lipids than the other two formulas because it will be simpler to break down smaller amounts of solid lipids than larger ones.
A system's degree of uniformity is indicated by its PDI; the more uniform the particle distribution in a mono-dispersion system, the lower the PDI value.[15] The NLC system's use of solid lipids serves to reduce the occurrence of molecular diffusion processes in NLC, resulting in increased retention and chemical stability of bioactive components. In contrast, liquid lipids function to enhance the efficiency of encapsulating bioactive compounds.[16] The cetyl palmitate and olive oil (8:3) in the NLC delivery system resulted in particles with a size less than 100 nm and remained stable after 45 days of storage. Therefore, cetyl palmitate was chosen.[17] Since nyamplung oil is a vegetable oil and has a fatty acid composition with 48.49% oleic acid, it was chosen as a solid lipid in this system. Because oleic acid can slow down the crystallization process and alter the rate at which the active chemicals in the NLC system release, it has the advantage of not being readily oxidized by the crystallization process when the temperature of the NLC system drops.[8]

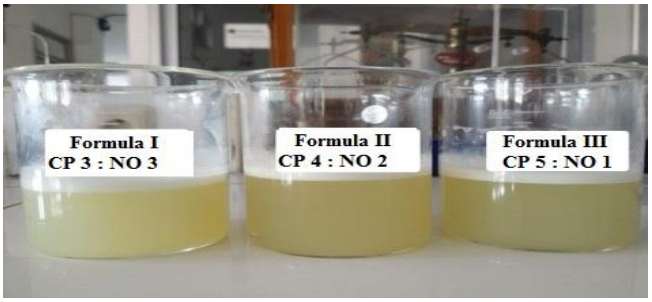

Figure 4: The physical appearance of formulated nanostructured lipid carrier (NLC) system using cetyl palmitate (CP) and nyamplung oil (NO).

Table 2: Characterization of formulated nanostructured lipid carriers.

Formula	Solid Lipid	Liquid Lipid	Particle Size (nm)	Polydispersity Index	Viscosity (dPas)	pH
FI	CP 3	NO 3	221.97 ± 5.03	0.250 ± 0.06	0.43 ± 0.05	5.39 ± 0.008
FII	CP 4	NO 2	214.70 ± 99.32	0.400 ± 0.009	0.73 ± 0.05	5.52 ± 0.008
FIII	CP 5	NO 1	215.70 ± 8.16	0.220 ± 0.04	0.36 ± 0.09	5.37 ± 0.008
FIV	SA 3	NO 3	393.94 ± 173.29	0.481 ± 0.234	266.56± 22.50	5.15±0.02
FV	SA 4	NO 2	897.27 ± 578.94	0.470 ± 0.703	807.53 ± 4.48	5.34 ± 0.009
FVI	SA 5	NO 1	2089.21 ± 46.25	0.592 ± 856	2089.21 ± 46.25	5.21 ± 0.015

A magnetic stirrer was used in the emulsification process to create NLCs, which were then followed by a HSH. The use of HSH has been known to produce nano-sized NLC formulas.[18,19] A HSH works by rubbing and breaking particles in a mixture of the NLC system. The absence of phase separation and creaming,[20] as well as the attainment of the desired particle size and a reasonably stable NLC, demonstrated the success of this NLC system. The physicochemical and functional stability of the emulsion-based carrier system can be controlled by designing the formulation's composition and conditions to produce stable NLC with a desired nanoparticle size.[21,22] The pH of the NLC measurement in Formulas I–VI was similar to each ingredient's acidic and inert pH. The skin has a pH range of 4–6, and the variations in NLC

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formulation concentrations showed no significant ($p \geq 0.005$) differences in pH values.

The viscosity of the NLCs tends to increase when a higher proportion of solid lipid relative to liquid lipid is used during its preparation (Table 2).[1] Despite having the same proportion of liquid and solid lipids, Formula I (3:3) has a higher viscosity than Formula III (5:1), which also contains solid lipids. However, this trend was not supported by the results of the three formulations. The higher viscosity observed may be attributed to the instability of either the liquid or solid lipids or to the effect of a higher concentration of solid lipids, which could alter the internal structure and consequently affect the viscosity. The viscosity of the preparation was determined using a viscosity test. A preparation will be more viscous if its viscosity value is higher, and vice versa. A system's stability rises as its viscosity value does. The viscosity of the liquid and solid lipid NLC systems is influenced by their composition; surfactants can help increase the stability of the value within the system. Formula IV (SA 3:NO 3) had a higher viscosity value than Formula V (SA 4: NO 2) and Formula VI (SA 5: NO 1), according to the results (Figure 5; Table 2) of the viscosity test conducted on the formulas. The viscosity value increased with the fat concentration[1]. While Formula IV (SA 3: NO 3) has the same percentage of both liquid and solid lipids, Formula VI (SA 5: NO 1) had more solid lipids than liquid lipids.

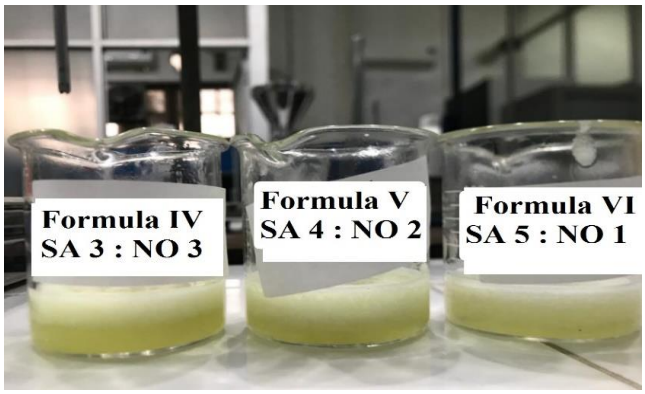

Figure 5: The physical appearance of formulated nanostructured lipid carrier (NLC) system using stearic acid (SA) and nyamplung oil (NO).

Solid lipids have a major influence on the system's stability. The value of particle size can also be impacted by viscosity values when utilizing bigger solid lipids. This could happen because a higher concentration of solid lipids can change the structure, which changes and affects the viscosity. The instability of liquid or solid lipids could also be the cause. The particle size obtained from Formulas I–III was less than 1000 nm (Table 2). This shows that the three formulas' particle sizes fall within the 10–1000 nm particle size range of the NLC system. According to the three formulas' results, Formula II (CP 4: NO 2) had the sample with the smallest particle size. Theoretically, Formula I (CP 3: NO 3) will result in a smaller particle size because, when compared to the other two formulas, it contains fewer solid lipids, making it easier to break down smaller amounts of solid lipids than larger ones. The viscosity of the system can also influence particle size, as higher viscosity often leads to larger particle formation. Variations in the concentrations of the system's components may further affect particle size due to differences in stirring speed or duration. The discrepancy between the experimental and theoretical results could be attributed to inadequate stirring time during the HSH (Ultra-Turrax) process, particularly if the results show that Formula 1 (CP 3: NO 3) exhibits the largest particle size compared to the other two formulations. Formula IV (SA 3: NO 3) exhibited the smallest NLC particle size. According to Zhang *et al*. (2011),[24] NLC particle sizes typically range from 10 to 1000 nm. In contrast, Formula VI (SA 5: NO 1) had a particle size of 2,089 nm, which exceeds the typical NLC range. This discrepancy may be attributed to the instability of the system containing solid lipids, which tend to form larger particles compared to liquid lipids. In theory, Formula IV (SA 3: NO 3) is a good formula since it has fewer solid lipids than Formulas V (SA 4: NO 2) and III (SA 5: NO 1). Compared to larger solid lipids, fewer solid lipids can result in a good system. Because Formula VI (SA 5: NO 1) requires less stirring time during the Ultra-turrax HSH process, it may produce the largest particle size when compared to the other two formulas. The results that do not align with the expected theory may be influenced by the system's viscosity, which can impact particle size. Additionally, variations in the concentration of the system's components and the stirring speed can also affect particle size outcomes. The particle size of most NLC formulations met the required range of 100–1000 nm. However, Formula VI exceeded 1000 nm. This larger size is likely due to the use of nyamplung oil at a low concentration, which, in combination with stearic acid, forms droplets with a larger diameter. In contrast, the combination of cetyl palmitate with nyamplung oil, even at a low liquid lipid concentration, produces particles around 200 nm.

The PDI indicates how uniform a system is; the more uniform the particle distribution in a monodispersed system, the lower the PDI value.[15] Samples with a PDI value close to 0 indicate a monodispersed sample, while a PDI value <1 indicates a polydispersed sample.[23] The analysis of the PDI for Formulas I–III indicates that these formulations are monodispersed. A monodispersed system exhibits a narrow particle size distribution, suggesting that the NLC system has a high degree of uniformity and homogeneity. The average particle size and PDI are critical parameters, as they determine the characteristics of the NLC system and influence its physical stability.[24] In general, NLC particle sizes range from 10 to 1000 nm.[25] For Formulas IV–VI, the PDI values were close to 0, indicating a monodispersed system. In contrast, PI values greater than 0 but less than 1 indicate a polydispersed sample. The results for Formulas IV–VI show a narrow particle size distribution, reflecting a high level of uniformity and homogeneity in the system.

Conclusion

The physical characteristics of NLCs in Formulas I–III were favourable, with all parameters falling within the expected ranges. These formulations exhibited pH values suitable for the skin, appropriate viscosity, particle sizes below 1000 nm, and a monodispersed system. Formulas IV–VI also showed generally good physical characteristics; however, the particle size in Formula VI exceeded 1000 nm. Overall, the NLC system demonstrated favorable characteristics, making it suitable as a drug carrier. These findings may serve as a foundation for future optimization and the development of NLC-based formulations for targeted drug delivery, particularly in cosmeceutical and topical therapeutic applications using natural bioactive compounds and locally sourced lipids like nyamplung oil.

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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