International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 5, 2023

Original Article

THE DEVELOPMENT FORMULATION OF *ELEUTHERINE PALMIFOLIA* EXTRACT-LOADED SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) USING D-OPTIMAL MIXTURE DESIGN APPROACH

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Received: 21 Feb 2023, Revised and Accepted: 18 Jun 2023

ABSTRACT

Objective: The develop a delivery system for extracting *E. palmifolia* as a model of medicinal ingredients in SNEDDS using a D-optimal design approach.

Methods: D-optimal mixture design optimizes *E. palmifolia* loaded SNEDDS by selecting SNEDDS composition as an independent factor (X) and characterizing SNEDDS as a response (Y). SNEDDS characterization in the optimal formula includes transmittance, emulsification time, pH, viscosity, particle size, and particle morphology. After obtaining one optimal formula, stability testing compares the initial characteristics (day one) with those of *E. palmifolia*-loaded SNEDDS.

Results: The SNEDDS was after storage for three months, namely day 30, day 60, and day 90. Miglyol 812, Tween 80, and polyethylene glycol (PEG) 400 were selected as oil, surfactant, and co-surfactant phases because they had the highest ability to dissolve *E. palmifolia* extract. The formula design with the D-optimal mixture design approach formulated *E. palmifolia* loaded SNEDDS with Miglyol 812, Tween 80, and PEG 400 components at an oil concentration of 2.13%, surfactant 5.81%, and co-surfactant 2.06% with stable characteristics in the storage period of 3 mo. Transmittance results in 96.75-98.74%, emulsification time 19.21-22.77 seconds, pH 6.69–7.71, viscosity 43.97-45.99 (cP), particle size 19.14-22.19 nm, spherical particle morphology.

Conclusion: The optimal formula for SNEDDS extract of *E. palmifolia* using the D-optimal design approach has physical and chemical characteristics that follow the SNEDDS specifications that have been determined.

Keywords: D-optimal mixture design, E. palmifolia, Self-nano emulsifying, SNEDDS

DOI: https://dx.doi.org/10.22159/ijap.2023v15i5.47645. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

In the process of discovering new pharmaceutical therapies, pharmaceutical formulation technology and drug delivery systems play an important role. The process of developing a drug delivery system generally takes a long time because many variables must be optimized to get a formula according to predetermined specifications. Therefore, experimental design using design expert®12 software trial with a D-optimal design approach can be used as a solution to reduce research time and costs to find the optimal formula design [1].

The technology of pharmaceutical preparation formulations and drug delivery systems plays an important role when discovering new pharmaceutical therapies [1].

The synthesis of active compounds or mixtures of natural ingredients is one of the discoveries of new drugs. Natural ingredients are selected because they contain a bioactive compound that can provide pharmacological effects. However, previous research estimated that 40% or more of natural compounds have low water solubility [2]. Low solubility in water and the lack of permeability to penetrate the absorption barrier can affect the bioavailability of a natural compound in the body [3]. The manufacture of pharmaceutical preparations based on drug delivery systems can be an alternative in manufacturing pharmaceutical preparations containing natural ingredients. *Eleutherine palmifolia (E. palmifolia)* can be one of the medicinal plants through the developed process as a new medicine.

E. palmifolia is a Kalimantan endemic plant, Indonesia. The community used *E. palmifolia* as a medicinal plant. The compounds contained in *E. palmifolia* extract are plants that have low water solubility. *E. palmifolia* contains naphthalene, anthraquinone, and naphthoquinone [4, 5]. The chemical compounds in *E. palmifolia* have anticancer bioactivity. Therefore, we developed a formulation technology to increase the bioavailability of *E. palmifolia* extract. Nanoparticle technology has now become a model in the development of drug delivery systems. Drug delivery systems are needed to facilitate the dispersion of lipophilic bioactive components in hydrophilic systems [6, 7]. Oil in water (O/W) emulsion-based, as a carrier system, is a very suitable way for encapsulation, protection, and carrying water-insoluble bioactive components. O/W is selected for pharmaceutical preparation and application to increase their solubility, stability, acceptability, and bioactivity [7].

Water-insoluble *E. palmifolia* extract was formulated in a self nanoemulsifying drug delivery system to increase solubility and oral bioavailability. Self nanoemulsifying drug delivery system (SNEDDS) is a homogeneous complex system consisting of oil, surfactants, and co-surfactants. The mixtures are thermodynamically stable. The composition and concentration of the formula determine the success of the formation of SNEDDS. The oil in the self nanoemulsifying drug delivery system formula will affect the droplet size and stability of the nanoemulsion formed [7]. To get the optimal formulation from *E. palmifolia* loaded SNEDDS, optimising the formula using a D-optimal mixture design will be necessary. This approach is the optimization of a process with the responses and results in several variables. The aim is to optimize the results by determining an ingredient that makes a formulation more optimal. The application of statistical experimental design has become a valuable tool in designing and optimizing pharmaceutical formulations and processes. It can investigate the effects of formulation variables and their interaction effects on desired characteristics [8]. Experimental design approaches are practical for optimizing the selection of SNEDDS andrographolide formulas with the simplex lattice design [9].

SNEDDS also is suitable for fisetin and gemfibrozil with the Box-Behnken design [10]. The results of this study are expected to improve the development of *E. palmifolia* extracts loaded with SNEDDS as a candidate oral drug.

MATERIALS AND METHODS

Extract preparation

The *E. palmifolia* was extracted three times with 500 ml ethanol at ambient temperature through sonication Q2400 (Sonica, USA) at 10 min intervals. About 25 grams of the sample was dissolved in 500 ml of 96% ethanol at 1:20. The filtrate was then separated from the solvent using a rotary evaporator (Heidolph, Germany).

Herbal material

The *E. palmifolia* samples were purchased from vendors in East Kalimantan and identified at the Materia Medica in Batu, East Java, Indonesia, with the accession number 074/342A/102.7/2018. The specimens were then stored in the pharmacognosy Laboratory of the Pharmacy Department, Maulana Malik Ibrahim, State Islamic University of Malang.

Formula material

Miglyol 812 purchased was from Sigma Aldrich, while Tween 80 and PEG 40 were from Merck.

Oil, surfactant, and co-surfactant selection screening

Our previous studies were the basis of the oil, surfactants, and co-surfactants selection. Various oils can be used during SNEDDS by producing high extract solubility and proper characteristics. The previous research focused on the effects of using vegetable oil in the formulation of *E. palmifolia*-loaded SNEDDS using the HLB approach (Annisa *et al.*, 2020). This study aimed to optimize the *E. palmifolia* extract solubility in various types of vegetable oil. We also optimized the SNEDDS formulation with the HLB approach. Based on the previous research, there was no stable formula in testing its stability (Annisa *et al.*, 2020). Based on the shortcomings of the research results, it was continued with *E. palmifolia*-loaded SNEDDS using Miglyol 812 (Annisa *et al.*, 2020). The results showed high solubility using Miglyol 812 oil and good characteristic on *E. palmifolia*-loaded SNEDDS using the hydrophilic-lipophilic balance (HLB) approach. The optimal result has a good sign and produces a stable formulation in storage for three months as indicated by no change in the initial characteristics of *E. palmifolia*-loaded. *E. palmifolia*-loaded SNEDDS with Miglyol 812 was chosen in this study using a different formulation approach.

E. palmifolia-loaded formula design self nanoemulsifying drug delivery (SNEDDS) using D-optimal mixture design approach

Formula optimization

Formula optimization was using design expert®12 software trial by selecting the D-optimal mixture design. Independent variables were along with the upper and lower limits of each variable, namely oil (1-7%), surfactants (1-8.5%), co-surfactants (0.5-8.5%) end *E. palmifolia* (50 mg). We also included the desired responses, namely transmittance, and emulsification. These two responses are the primary responses in determining the optimal formula. Percent transmittance is within the result of 80-100%, and the emulsification time is within the range of 1-120 seconds. The design expert®12 software trial analyses and produces the optimum formula solution according to the criteria and desirability index value. The result shows the prediction results of the design expert®12 software trial that are the expected criteria. After obtaining the optimal formula, we proceed with the optimal formula characterization, including transmittance, emulsification time, pH, viscosity, particle size, particle morphology, and stability.

Verification of the optimum formula

The verification test carried out includes the percent transmittance and emulsification time. This re-testing is selected to determine and ensure the optimum formula results from the D-optimal mixture design by the actual conditions. Verification of the optimum formula was done using statistical analysis of variance (ANOVA) with a confidence level of 95%. Experimental results and predictions are considered not significantly different if the significance is>0.05.

Preparation of E. palmifolia-loaded self nanoemulsifying drug delivery (SNEDDS)

The optimal SNEDDS formula design was added 50 mg of *E. palmifolia* extract, then mixed until homogeneous with a magnetic stirrer with a speed of 300 rpm for 10 min. The preparation obtained is called preconcentrate and stored at 25 °C for further characterization.

Characterization of *E. palmifolia*-loaded self-nano emulsifying drug delivery (SNEDDS)

The characterization of the optimal SNEDDS formula for *E. palmifolia* extract was carried out at several testing times. In the first 1 x 24 h after the manufacturing process, this characteristic is marked by the first day of testing. After that, characteristic testing was carried out after storage on the 30th, 60th, and 90th days.

Transmittance

The emulsion formed in the previous stage was observed using a spectrophotometer UV 1800 (Shimadzu, Japan) at a wavelength of 650 nm. If the sample transmittance percentage results are close to that of distilled water, which is 100%, it is assumed that the nanoemulsion droplets have been nanosized. Three repeated readings were performed for the sample [12].

Emulsification time

The emulsification time was calculated for the *E. palmifolia* SNEDDS in two media, namely artificial gastric fluid pH 1,2±0,5 without pepsin and artificial intestinal fluid pH 6,8±0,5 without pancreatin. The composition of AIF and AIF (table 1). Usually, self-emulsification is the time required by the pre-concentrate to form a homogeneous mixture on dilution when the disappearance of SNEDDS is observed visually. During this process, 100 µl of each formulation was added dropwise to the 200 ml media with a stirrer (Heidolph, Germany) at a speed of 100 rpm at 37±0,5 °C. The time required for the disappearance of the SNEDDS was recorded. Three repeated readings were performed for the sample [12].

Table 1: Formulation of artificial gastric fluid (AGF) and artificial intestinal fluid (AIF) for emulsification time test

Formulation AGF pH 1.2±0.5		Formulation AIF pH 6.8±0.5	
NaCl	20.00 mg	$MgCl_2$	0.15 g
HCl 37%	0.70 ml	$CaCl_2$	0.15 g
Water distillate	ad 100.00 ml	KCl	0.09 g
		NaCl	1.76 g
		$NaHCO_3$	0.42 g
		Water distillate	ad 500 ml

pH measurement

The pH of each formulation was measured using a pH meter S220 (Mettler Toledo, USA). The pH meter electrodes were inserted into 10.00 ml of *E. palmifolia* SNEEDS, and the number indicated by the pH meter was recorded. Three repeated readings were performed for the sample [12].

Viscosity

The viscosity testing was conducted using a cone and plate viscometer (Brookfield, USA). The stationary plates (CP-40) were filled with 0.50-2.00 ml *E. palmifolia* SNEDDS and placed in the sample cup. This is to ensure it is bubble-free and evenly spread. The sample cup was also reassembled on the viscometer, turned on, and

then left until the reading was stable. Three repeated readings were performed for the sample [12].

Particle size analysis

The particle size of the emulsion formed after the reconstitution of SNEDDS was determined by dynamic light scattering Nanowave II (Microtrac, USA). The formulations were diluted at a ratio of 1:10 *w/w* with water and mixed well for 1 min. The diluted samples were transferred into cuvettes (model nano PTFE). A relative refractive index of 1.20 (ratio of the index between the oil and water phases) was used. Three repeated readings were performed for the sample [12].

Particle morphology

Particle morphology was measured using Transmission Electron Microscopy (TEM JEOL JEM 1400, USA). The morphological analysis was conducted by dropping the sample on a 400 mesh Cu grid previously coated with a film from Formvar and carbon. The mixture was allowed to dry and analyzed at 100 KVA at several points with various magnifications. The results of the morphology formed were observed and recorded.

Stability studies

Heating-cooling cycle

The heating-cooling cycles were performed three times at temperatures between 4 °C±2 °C and 45 °C±2 °C, each stored for a minimum of 48 h. The formulation which survived these temperatures without cracking, creaming, phase separation, coalescence, or phase inversion was selected for the freeze-thaw stress test. These were diluted with double distilled water (1:25), and the resulting nanoemulsion was observed for instability problems [12-14].

Freeze-thaw cycle

The freeze-thaw test included three cycles in a temperature range of −20 °C±2 °C to 25 °C±2 °C stored for at least 48 h. The formulation was diluted with double distilled water (1:25), and the resulting nanoemulsion was observed for instability problems [12-14].

RESULTS AND DISCUSSION

Optimization and verification of the optimal formula

The selection of the right components is a prime condition for formulating stable SNEDDS preparations. The initial stage includes the independent variables with each variable's upper and lower limits, namely oil, surfactant, co-surfactant, and response (transmittance and emulsification time). Based on this, 17 run formulas were formulated and analyzed for feedback. The details of the run formula and the results of the optimization response are presented in table 2.

The analysis of the response to the optimization formula was carried out on the transmittance and emulsification time. Based on the analysis of the approaching model carried out in this study, it is known that the approaching model obtained is linear. The statistical analysis used was variance (ANOVA) analysis with a confidence level of 95%. The transmittance test shows that the linear model is significant with a p-value of 0.0013, and the Lack of Fit is insignificant with a p-value of 0.1690. Meanwhile, the emulsification time shows a significant linear model with a p-value of 0.0133 and an insignificant Lack of Fit with a p-value of 0.1688. The ternary diagram results of the responses are presented in fig. 1.

Table 2: Optimization formula of *E. palmifolia*-loaded self-nano emulsifying drug delivery

Runs (formula)	Independent variables (X) component of formula			Response (Y) characteristics of SNEDDS	
	Miglyol 812 (%)	Tween 80 (%)	PEG 400 (%)	Transmittance (%)	Emulsification time (second)
1	5.53	3.75	0.71	59.97	114.40
2	3.36	3.52	3.12	92.27	31.70
3	7.00	1.94	1.06	83.31	87.50
4	4.46	1.00	4.54	58.61	121.00
5	5.23	2.16	2.61	62.08	30.10
6	4.00	5.50	0.50	93.99	165.80
7	2.72	1.00	6.28	78.24	12.00
8	3.36	3.52	3.12	94.76	18.40
9	1.00	8.50	0.50	97.69	168.20
10	1.00	2.92	6.08	99.81	20.00
11	1.78	6.18	2.04	96.60	34.40
12	1.00	4.68	4.32	98.98	38.20
13	1.00	1.00	8.00	98.50	17.50
14	7.00	1.94	1.06	58.44	36.50
15	3.36	3.2	3.10	89.22	23.70
16	4.46	1.00	4.54	57.49	28.60

The yellow-shaded area is an area that can form a clear and stable nanoemulsion without any phase separation. The site has been tested for transmittance and emulsification time. Meanwhile, the grey part of the image is an area that produces an unstable nanoemulsion characterized by separating the two phases and becoming cloudy. Table 1 shows that the higher the oil concentration (Miglyol 812). The nanoemulsion formed does not result in high transmittance results and fast emulsification time. Therefore, it can identify that the nanoemulsion formed is unstable. The stability occurs because the surfactants and co-surfactants cannot reduce the interface stress of Miglyol 812 in large quantities. Increasing the amount of Tween 80 can improve the stability of the nanoemulsion formed as it can reduce the interface tension of the two fluids with PEG-400.

Optimization of D-optimal mixture design produces SNEDDS with transmittance response between 80–100% and emulsification time<120 seconds. The optimal response was obtained from the composition of Miglyol 812, Tween 80, and PEG 400 with 50 mg of *E. palmifolia* in each formula. From the results of the D-optimal mixture design optimization, the optimal formula is determined with predetermined criteria. The optimal formula obtained was regenerated with three replications and characterizations. The optimal formula has a composition of Miglyol 812 (2.13%), Tween 80 (5.81%), and PEG 400 (2.06%) concentration. The preparation is made in 10 ml with content of *E. palmifolia* as much as 50 mg.

We chose to minimize the Miglyol 812 criterion because the lower the oil concentration, the better the visual appearance. High oil concentration will cause a decrease in nanoemulsification and an increase in particle size. Therefore, the criteria to minimize was chosen to produce a small particle size and maximize nanoemulsification. Tween 80 also selected the criteria to minimize and reduce surfactant toxicity and minimize digestive tract irritation. PEG 400 was chosen as the maximization criterion because the co-surfactant was used to help the performance of the surfactant in reducing the fluid interface stress, so it needed a high concentration to maximize the performance of the co-surfactant. The

analysis was carried out based on the above criteria using the design expert®12 software trial to obtain an optimal formula with the predictive response that has been prepared. The optimal formula and prediction results can be seen in table 3.

The objective of the formula optimization for pharmaceutical preparations, including SNEDDS generally, is to determine the variable level of a strong product with high-quality characteristics produced. After that, the optimal formula result is characterized. The measurement results obtained are then compared with the predicted values obtained from the design expert®12 software trial.

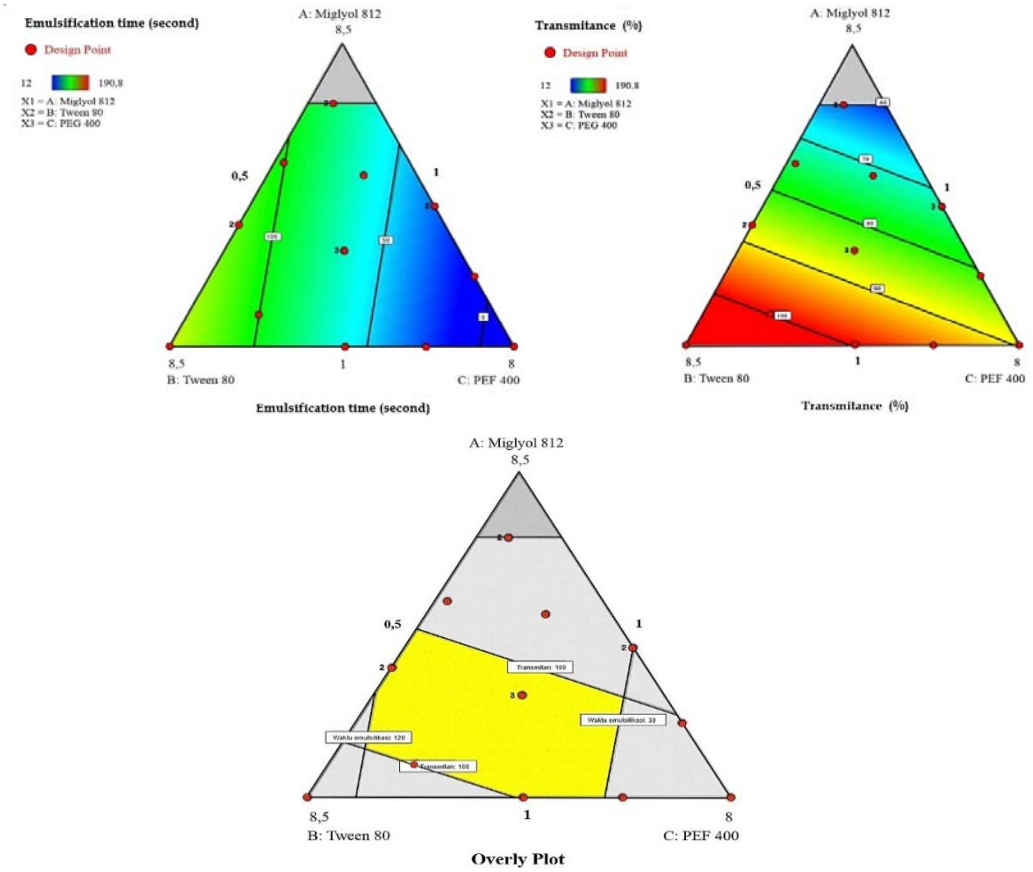


Fig. 1: Ternary phase diagram of Miglyol 812, Tween 80, and PEG 400

Table 3: Comparison of the predicted value with the experimental value

Parameter	Predicted value	Experimental value	Interpretation
Transmittance (%)	81.65–105.00	96.75±0.53	in range
Emulsification Time (second)	37.64–61.64	43.22±2.42	in range

All values as mean±standard deviation (n=3).

Characterization of *E. palmifolia*-loaded self-nano emulsifying drug delivery (SNEDDS)

Transmittance

The results of transmittance can be seen in table 4. Based on the results of multivariate satistical analysis (MANOVA) using SPSS 25 software, the p-value is 0.983>0.05, meaning there is no difference in the transmittance on days 1, 30, 60, and 90 of the SNEDDS formula of *E. palmifolia* and SNEDDS without *E. palmifolia*. It indicates that *E. palmifolia* loaded with SNEDDS can produce stable SNEDDS. SNEDDS with a transmittance value above 80% will generate a clear and transparent solution. The transmittance value obtained in this study was 96.75- 98.74% with a clear compound. These results provide an early indication of no aggregation or particle growth even after 24 h of dilution. The transmittance percentage close to 100% is a droplet size indication in the nanometer range [15]. This condition relates to the SNEDDS droplet size that disperses into the water with a high transmittance value.

Table 4: The transmittance results of the SNEDDS formula test

Formula	Transmittance (%)			
	Day 1	Day 30	Day 60	Day 90
SNEDDS *E. palmifolia* extract	95.1±0.50	97.50±1.68	97.40±1.13	97.14±0.47
SNEDDS without *E. palmifolia* extract	96.75±0.53	98.74±0.11	98.33±0.40	98.06±0.09

All values as mean±standard deviation (n=3).

Emulsification time

The results of emulsification time can be seen in table 5. The multivariate statistical analysis (MANOVA) using SPSS 25 software shows that the p-value is 0.970>0.05. It means that there is no difference in the emulsification time of testing on days 1, 30, 60, and 90 in AIF and AGF media on the SNEDDS formula for *E. palmifolia* extract and SNEDDS without *E. palmifolia* extract. Emulsification time testing was carried out to see the process and time required for nanoemulsion formation *in vitro* by visual observation. The test results show that all formulas meet the criteria for testing the emulsification time. It indicates the SNEDDS preparation to form a transparent nanoemulsion system quickly when it enters the digestive tract. The formation of a nanoemulsion system on SNEDDS preparations dispersed in aqueous media (AIF and AGF) occurs due to surfactants and co-surfactants in the formula and during the stirring process, which is finally able to form a dense interface layer in a nanoglobul system [15, 16].

Table 5: The emulsification time results of the SNEDDS formula test

Formula	Emulsification time (sec)							
	Day 1		Day 30		Day 60		Day 90	
	AIF	AGF	AIF	AGF	AIF	AGF	AIF	AGF
SNEDDS *E. palmifolia* extract	22.33±1.64	23.18±1.11	22.89±0.88	22.36±0.75	22.77±1.78	22.27±5.25	22.46±1.02	22.21±0.76
SNEDDS without *E. palmifolia* extract	22.41±0.56	19.21±0.15	22.41±0.56	19.21±0.15	22.10±1.93	19.81±0.61	22.77±3.54	19.50±0.43

All values as mean±standard deviation (n=3).

pH measurement

The results of pH can be seen in table 6. Based on the multivariate statistical analysis (MANOVA) results using SPSS 2 software, it is known that the p-value is 0.064>0.05. It means that there is no difference in pH on days 1, 30, 60, and 90 of the SNEDDS formula for *E. palmifolia* extract and SNEDDS without *E. palmifolia* extract.

Viscosity

The results of viscosity can be seen in table 7. Based on the multivariate statistical analysis (MANOVA) results using SPSS 25 software, it is known that the p-value is 0.961>0.05. It means there is no difference in the viscosity on days 1, 30, 60, and 90 of the SNEDDS formula for *E. palmifolia* extract and SNEDDS without *E. palmifolia* extract.

Table 6: The pH measurement results of the SNEDDS formula test

Formula	pH measurement			
	Day 1	Day 30	Day 60	Day 90
SNEDDS *E. palmifolia* extract	7.71±0.61	6.84±0.16	6.70±0.26	6.69±0.19
SNEDDS without *E. palmifolia* extract	7.70±0.10	7.40±0.35	7.40±0.20	7.37±0.28

All values as mean±standard deviation (n=3).

Table 7: The results of the SNEDDS formula viscosity test

Formula	Viscosity (cP)			
	Day 1	Day 30	Day 60	Day 90
SNEDDS *E. palmifolia* extract	45.48±0.33	45.99±0.59	45.32±2.00	45.03±1.81
SNEDDS without *E. palmifolia* extract	44.36±2.24	44.32±2.49	43.97±2.46	44.32±0.22

All values as mean±standard deviation (n=3).

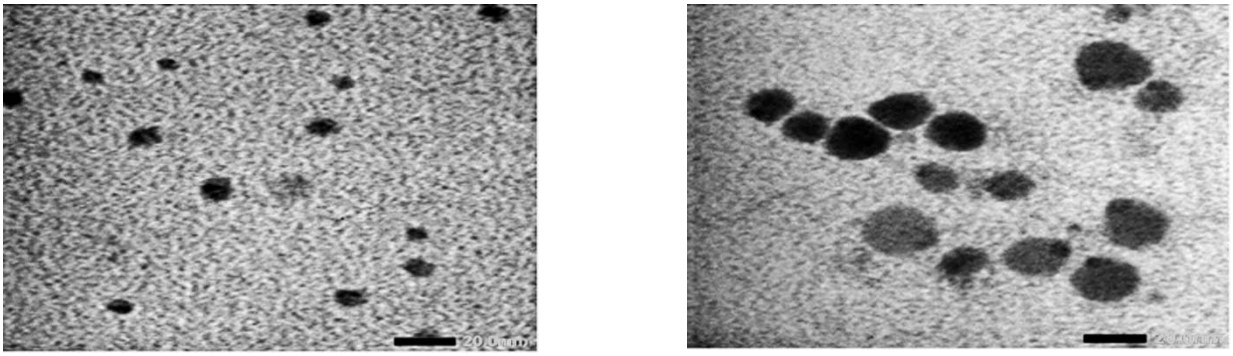
Particle size

The results of particle size can be seen in table 8. The multivariate statistical analysis (MANOVA) using SPSS 25 software shows that the p-value is 0.521>0.05. There is no SNEDDS *E. palmifolia* particle size and SNEDDS without *E. palmifolia* testing on days 1, 30, 60, and 90 on the AIF and AGF media. The particle measurements show that the results are still within the SNEDDS size range, namely 10–200 nm. The resulting particle size will affect the bioavailability of the drug in the body. The small particle size will have a larger surface area, which will speed up digestion by enzymes so that the drug can be released more easily for absorption. Increasing the amount of oil used in the formulation will increase the particle size due to decreasing the number of surfactants and co-surfactants used. Conversely, increasing the amount of surfactant and co-surfactant used will reduce the particle size due to increased emulsifier adsorption around the droplet interface and interface stress in the system [10]. *E. palmifolia* is lipophilic in a preconcentrated form consisting of extracts, oils, surfactants, and co-surfactants. This preconcentrate will form a homogeneous nanoemulsion in the nanometer range after being diluted with aqueous media. The drug remains dissolved in nanometer-sized nanoemulsion droplets and is absorbed into the systemic circulation via lymphatic mechanisms. Smaller droplet sizes lead to a more extensive interface surface area for drug absorption [10, 18, 19].

Table 8: The particle size results of the SNEDDS formula test

Formula	Particle size (nm)							
	Day 1		Day 30		Day 60		Day 90	
	AIF	AGF	AIF	AGF	AIF	AGF	AIF	AGF
SNEDDS *E. palmifolia* extract	22.19±0.14	20.17±0.57	20.92±1.86	19.88±1.65	20.60±0.36	19.46±2.19	20.45±0.39	19.53±0.75
SNEDDS without *E. palmifolia* extract	19.14±1.31	20.00±0.65	19.40±1.39	19.59±1.35	19.39±4.58	20.23±2.19	19.76±2.59	19.97±3.19

All values as mean±standard deviation (n=3).



A B

Fig. 2: Particle morphology (A) *E. palmifolia* loaded SNEDDS (B) SNEDDS without *E. palmifolia* using transmission electron microscopy (TEM) at a magnification of 40.000 times

Particle morphology

Tests were carried out using Transmission Electron Microscopy (TEM) to visually determine nanoemulsions' morphology. Fig. 2 shows the observation results. The globule is spherical, homogeneous, and dispersed, with a size that meets the nanometric criteria for nanoemulsion formulations.

Stability studies

The results of the stability study can be seen in table 9. The last stage of testing is a thermodynamic test aimed at determining the stability of SNEDDS preparations. The results show that all SNEDDS preparations have good physical stability thermodynamically. Heating cooling and freeze-thaw tests were carried out to see the resistance of SNEDDS from separation and deposition due to temperature changes [20]. Based on the stability test results, it is known that the SNEDSS formula shows good physical characteristics and stability. The particle size after SNEDDS mixes with gastrointestinal fluids is essential because it affects drug release and absorption rate. The small particle size will increase the surface area, lead to faster absorption, and increase bioavailability. The small size of the globules can be attributed to the appropriate mixture of surfactants and cosurfactants. It provides an adequate reduction in free energy to counter thermodynamic instability.

Table 9: The thermodynamic stability results of the SNEDDS formula test

Formula	Heating-cooling cycle (4 °C±2 °C] and 45 °C±2 °C)	Freeze-thaw cycle (−20 °C±2 °C and 25 °C±2 °C)
SNEDDS *E. palmifolia* extract	Stable	Stable
SNEDDS without *E. palmifolia* extract	Stable	Stable

DISCUSSION

Self nanoemulsifying drug delivery system (SNEDDS) is an isotropic mixture of oil, surfactant, and co-surfactant. The prime characteristic of SNEDDS is the ability to form nanoemulsions. The peculiarity is regarding gastrointestinal fluid in light-stirring of gastrointestinal peristalsis [18, 21]. However, the mechanism for self-emulsion has not been explained widely. It can occur when the entropy changes because the dispersion is greater than the energy required to increase the surface area [22]. The free energy of conventional emulsion formulas is a direct function of the binding energy to form a new surface between oil and water.

The SNEDDS formula's components greatly influence the SNEDDS formation's success. The role of the oil phase in the SNEDDS system is very prominent because it affects the ability to form nanoemulsions directly in a fast time, droplet size, drug solubility, and drug fate in the body [23, 24]. The oil with the maximum solubility potential for the selected drug model was the oil phase for the SNEDDS formulation. It can help to achieve peak drug loading in SNEDDS [25]. The oil used in this study was Miglyol 812. Miglyol 812 is a medium-chain triglyceride (MCT) widely used to prepare SNEDDS. MCTs have a higher solvent capacity than long-chain triglycerides and tend not to go rancid due to oxidation [26] and increased intestinal absorption. The advantages of MCT over LCT are each fatty acid's configuration, especially those with double bonds. The double bond strengthens the cis isomer configuration and bends the chain. It limits the fatty acid configuration [27]. Oils with too long hydrocarbon chains, such as soybean oil or long-chain triglycerides (LCT), are confounding to form nanoemulsions. It will create droplet sizes that are much larger than SNEDD systems using MCT.

Surfactants have an esteemed role in SNEDDS to reduce droplet size. They can stabilize active substances during a long period of storage at the absorption site, thus preventing deposition in the digestive tract. The surfactants' molecular structure has lipophilic and hydrophilic parts due to the polar part and non-polar parts. Surfactants affect the emulsification time of the SNEDDS formula. Surfactants can function in increasing the oil's ability to dissolve drugs. Water-soluble surfactants such as Tween 80 are often used in the SNEDDS formula. After all, it is safe, biocompatible, and relatively stable because it is not affected by pH changes. The type of surfactant used in the SNEDDS formula is a surfactant with an HLB value>10. It is because HLB>10 can form an oil-in-water (O/W) type nanoemulsion. A mixture of hydrophobic and hydrophilic surfactants can form nanoemulsions with the desired characteristics in some formulations [28].

Co-surfactant aims to help the work of surfactants in reducing the surface tension of water and oil. It increases the dissolution of active substances. The SNEDDS formulation's co-surfactant also increases the drug loading capacity in the SNEDDS system. The co-surfactant affects the emulsification time and droplet size of the nanoemulsion. The co-surfactant in this study is short-chain alcohol. It reduces interface stress and increases interface fluidity. Selected co-surfactant increases the mixing of water and oil because of their partition between two phases [28].

Surfactants and co-surfactants will increase the effectiveness of nanoemulsion system formation if they can mix well with the oil phase. The test results show that the surfactant Tween 80 and co-surfactant PEG 400 have a proper mix with *E. palmifolia* and Miglyol 812. The surfactants and co-surfactants in the nanoemulsion system work together to form a good and flexible interface system and reduce the surface tension value up to close to zero to support the formation of stable nanosized globules [29].

Theoretically, the nanoemulsion size is influenced by the ratio of surfactant to co-surfactant. The higher the ratio of surfactant to co-

surfactant, the smaller the size of the nanoemulsion obtained. The oil and surfactant composition influences the nanoemulsion's droplet size. Oil can increase the ability of SNEDDS to carry drugs, but it has the disadvantage that it can cause the size of SNEDDS to become more prominent. The ideal SNEDDS formula generally uses a smaller oil ratio than surfactants [12].

Thermodynamic stability studies were conducted to detect metastable formulas using the freeze-thaw and heating-cooling cycle methods. A thermodynamic stability test was used to confirm the prepared SNEDDS in a short time. The results of SNEDDS thermodynamic stability (table 9) showed stable results, namely no creaming, phase separation, coalescence, or phase inversion after storage at 4 °C±2 °C and 45 °C±2 °C (freeze-thaw cycle). And 20 °C±2 °C and 25 °C±2 °C (heating-cooling cycle) for 48 h for 3 cycles. Based on the thermodynamic stability test results, it can be concluded that the SNEDDS of the Dayak onion extract and the SNEDDS without the Dayak onion extract were thermodynamically stable.

CONCLUSION

The formula design with the D-optimal mixture design approach is suitable to formulate SNEDDS of *E. palmifolia* extract with Miglyol 812, Tween 80, and PEG 400 components at an oil concentration of 2.13%, surfactant 5.81%, and co-surfactant 2.06% by producing stable characteristics in the storage period of three months. Transmittance results 96.75– 98.74%, emulsification time 19.21 – 22.77 seconds, pH 6.69–7.71, viscosity 43.97 – 45.99 (cP), particle size 19.14–22.19 nm, spherical particle morphology.

ACKNOWLEDGEMENT

Thank you to the Department of Pharmaceutical Science, Faculty of Pharmacy, Airlangga University, Surabaya, and the Department of Pharmacy, Faculty of Medicine and Health Science, Maulana Malik Ibrahim State Islamic University, Malang for providing the laboratory facilities so that this research can be carried out well. This research is part of the research on the Doctoral Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Airlangga. Do not forget to also convey to Kementrian Agama Republik Indonesia, which has provided scholarship funds for the 5000 doctoral programs.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Concept–RA, MY, EH; Design–RA, MY, EH; Supervision–MY, EH; Resources–RA, MY, EH; Materials–RA, MY, EH; Data Collection and Processing–RA, RM, MY, EH; Analysis and Interpretation–RA, MY, EH; Literature Search–RA, MY, EH; Writing–RA; Critical Reviews RA, RM, MY, EH.

CONFLICT OF INTERESTS

The authors declared no conflict of interest.

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