

RESEARCH ARTICLE OPEN ACCESS

Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Ethyl Acetate Fraction of *Begonia medicinalis* Herb and Ethanol Fraction of *Moringa oleifera* L. Leaves

Muhammad Sulaiman Zubair¹  | Rahma Mutiani¹ | Evi Sulastr¹  | Armini Syamsidi¹  | Saipul Maulana¹  | Arwansyah Arwansyah² 

¹Pharmacy Department, Faculty of Sciences, Tadulako University, Palu 94118, Central Sulawesi, Indonesia | ²Department of Chemistry Education, Faculty of Teacher Training and Education, Tadulako University, Palu 94118, Central Sulawesi, Indonesia

Correspondence: Muhammad Sulaiman Zubair (sulaimanzubair@untad.ac.id)

Received: 23 August 2025 | **Revised:** 16 April 2026 | **Accepted:** 21 April 2026

Academic Editor: Arnab Biswas

ABSTRACT

The combination of ethyl acetate fraction of *Begonia medicinalis* and ethanol fraction of *Moringa oleifera* was developed into self-nanoemulsifying drug delivery system (SNEDDS) to increase the solubility of combined fraction. This study aims to determine the optimal concentrations of virgin coconut oil (VCO), Tween 80, and propylene glycol using the D-optimal mixture design (DMD) method on Design-Expert software and phytochemical contents and anticancer activity by the in vitro MTT method. Determination of the optimal formula was determined by using ANOVA statistical analysis between components of material and the response. The optimal formula was then chosen based on the desirability value close to 1. Formula optimization has resulted in nine formulas. The optimum formula was found at Formula 5 (13% VCO, 60% Tween 80, and 22% propylene glycol). The characteristic results showed that the optimum formula had a particle size of 17.3 ± 0.51 nm, a polydispersity index (PDI) of 0.17 ± 0.02 , an emulsification time of 25 ± 1.00 s, and a transmittance percentage of $85.11\% \pm 0.39\%$. Total phenolics, total flavonoids, and total saponins were found to be 54.90 ± 0.77 mg GAE/g, 43.85 ± 2.18 mg QE/g, and 271.27 ± 7.10 mg EE/g, respectively. The optimized formula was able to inhibit the proliferation of HeLa and T47D cancer cells with IC_{50} of $21.8 \mu\text{g/mL}$ and $117.3 \mu\text{g/mL}$, respectively. Therefore, the study showed that the DMD effectively described and predicted an optimum SNEDDS formula for the combined fractions.

1 | Introduction

Begonia medicinalis and *Moringa oleifera* are commonly used as medicinal plants to treat several diseases in Central Sulawesi, Indonesia. In silico studies have reported that alkaloid compounds, flavonoid glycosides, steroid glycosides, and triterpenoid glycosides in *B. medicinalis* have potential anticancer activity [1]. The methanol extract has demonstrated anticancer activity against breast cancer cells (T47D) and cervical cancer cells (HeLa) [2]. Several glycosides which were isolated from ethyl acetate fraction have shown potential cytotoxic activity against HCT-116 and MCF-7 cancer cells through an apoptosis induction

mechanism [3]. Studies have also reported that its ethyl acetate fraction acts as an immunostimulant, providing the highest stimulation to lymphocyte cells [4]. Additionally, it has demonstrated potential antioxidant and antiviral activity, with a half-maximal inhibitory concentration (IC_{50}) of $8.26 \mu\text{g/mL}$ [5].

Meanwhile, *M. oleifera* has been reported to possess diverse pharmacological activities, including anticancer, antimicrobial, antidiabetic, antifertility, antiasthmatic, anti-inflammatory, antihypertensive, and antioxidant activities, which are attributed to its high phenolic and flavonoid content [6–11]. The ethanol fraction contains the most effective antioxidant compounds due

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Copyright © 2026 Muhammad Sulaiman Zubair et al. *Journal of Nanotechnology* published by John Wiley & Sons Ltd.

to the higher dissolution efficiency of flavonoid compounds, making them more soluble in ethanol [12]. Our previous study showed that the combination of *B. medicinalis* and *M. oleifera* ethanol extract at a dose of 100:100 mg/kg body weight exhibited immunomodulatory activity by increasing the macrophage phagocytic activity and TNF- α and IFN- γ cytokine inductions [13].

Drug delivery systems play a crucial role in the discovery of pharmaceutical therapies. Studies have shown that nanometer-sized formulations are able to enhance the dissolution and oral bioavailability of several active compounds [14]. The absorption of active ingredients increases due to the improved solubility and larger surface area of the particles. Particle sizes in the range of 10–20 nm may inhibit the spread of cancer [15].

The development of pharmaceutical preparations in the form of a self-nanoemulsifying drug delivery system (SNEDDS) has great potential for formulations that contain hydrophobic ingredients. SNEDDS is an isotropic mixture of oil, surfactant, and co-surfactant that forms a nanoemulsion spontaneously when mixed with water through gentle agitation in the digestive tract [16]. Upon mixing with water, SNEDDS typically forms a nanoemulsion system with a globule size of less than 100 nm. Several studies on SNEDDS formulations have demonstrated the increased absorption and permeation of active substances by forming nanometer-sized particles [17].

To ensure a rational formulation strategy, the selection of SNEDDS components in this study was based on their physicochemical properties and compatibility with phytoconstituents. The combination of virgin coconut oil (VCO), Tween 80, and propylene glycol exhibited synergistic effects that facilitated efficient self-nanoemulsification formulation. VCO, a medium-chain triglyceride-rich oil, provides superior solubilization of phytoconstituents relative to many vegetable oils and has been widely used in SNEDDS formulations for herbal extracts. Tween 80 effectively reduced interfacial tension and promoted spontaneous O/W nanoemulsion formation, consistent with prior reports demonstrating its utility as a primary surfactant in SNEDDS. Propylene glycol contributes to cosolvency and enhances the flexibility of the interfacial film, enabling more uniform nanoemulsion formation. These properties have been repeatedly validated in high-impact SNEDDS studies involving natural products such as curcumin, silymarin, quercetin, and naringenin [18–20].

The optimization of nanoemulsions is necessary to determine the best formula with the desired product characteristics under specific processes and conditions. Moreover, numerous possible formula combinations require significant time and experimental effort to identify the most appropriate formulation approach. Therefore, it is essential to develop screening tools that enable successful formulation determination within a short time and with manageable experimental effort [21]. For this purpose, the use of data processing software offers a practical solution. Design-Expert software has been widely reported for its applications in drug delivery systems, such as slow-release tablets and systems involving liposomes, ethosomes, and nanoparticles. Furthermore, the use of a D-optimal mixture design (DMD) allows efficient exploration of the formulation space under constrained conditions, where the sum of component proportions must equal 100%. This approach provides a systematic and statistically robust method to identify the optimal

combination of components while minimizing experimental runs [22, 23].

Despite the growing interest in nano-based delivery systems for herbal medicines, research focusing on the combined formulation of fractions from *B. medicinalis* and *M. oleifera* in a SNEDDS system has not been conducted. Previous studies have developed SNEDDS preparations combining ethanol extracts of both plants, and the optimized formulation showed good characterization and possessed anticancer and immunomodulatory properties [24–26].

Therefore, the novelty of this study lies in the development of a combined SNEDDS formulation of two plant bioactive fractions, the application of DMD to efficiently optimize formulation variables, and the evaluation of anticancer activity of the optimized nanoformulation, providing insight into the relationship between formulation characteristics and biological performance.

2 | Materials and Methods

2.1 | Materials

Begonia medicinalis was obtained from Toddopuli, Soyo Jaya District, North Morowali, and *Moringa oleifera* was obtained from Sibedi Village, Sigi Regency, Central Sulawesi, Indonesia. Plant identification was carried out at the UPT. Sumber Daya Hayati, Tadulako University (voucher no. 253/UN.28.UPT-SDHS/LK/2024). HeLa (cervical adenocarcinoma; RRID: CVCL_0030) and T47D (breast ductal carcinoma; RRID: CVCL_0553) cell lines were obtained from a culture collection of the Laboratory of Parasitology, Gadjah Mada University, Yogyakarta. Distilled water, 70% ethanol, VCO, Tween 80, propylene glycol, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were acquired from Sigma.

2.2 | Extraction and Fractionation

B. medicinalis (leaves and stems) and *M. oleifera* (leaves) were extracted by maceration using 70% ethanol for 3–5 days. The collected filtrate was then evaporated by rotary evaporator until obtained the viscous extract. Ethyl acetate fraction of *B. medicinalis* was obtained by successive liquid–liquid extraction of ethanol extract using different solvents of n-hexane to ethyl acetate following the method in our previous research [27]. Meanwhile, ethanol fraction of *M. oleifera* was obtained by successive liquid–liquid extraction of ethanol extract using different solvents of n-hexane, dichloromethane, ethyl acetate, acetone, and ethanol following the previous research method [12].

2.3 | SNEDDS Preparation

The DMD method on Design-Expert was used to determine the concentration of oil, surfactant, and cosurfactant used in the formulation (Tables 1 and 2). Each fraction weighed 1:1 and was dissolved in ethanol. After that, the solution was mixed with VCO and stirred using a magnetic stirrer for 15 min (Mixture A). Next, propylene glycol was added to Mixture A while stirring with a magnetic stirrer for 15 min (Mixture B). Then, Tween 80 was added to Mixture B while stirring with a magnetic stirrer for 15 min until the mixture becomes homogeneous. Then it was sonicated for 30 min.

TABLE 1 | Component values used in DMD.

Component	Minimum (%)	Maximum (%)
VCO	12	22
Tween 80	60	70
Propylene glycol	12	22

2.4 | Particle Size and Polydispersity Index (PDI)

The size of the sample droplets was measured using a dynamic light scattering particle size analyzer (Malvern Instruments Ltd) at a wavelength of 633 nm and 25°C with a scattering angle of 90° [28]. The PDI is a measure of droplet size homogeneity and varies from 0.0 to 1.0. The higher the polydispersity, the lower the uniformity of droplet size in the formulation. The closer the polydispersity value is to zero, the more homogeneous the droplets are [29].

2.5 | Percentage of Transmittance

The SNEDDS formula was taken in an amount of 40 µL, then dissolved in 10 mL of distilled water, stirred until homogeneous, and read for transmittance using a UV-Vis spectrophotometer at λ 650 nm [30].

2.6 | Emulsification Time

Self-emulsification efficiency is assessed by using dissolution apparatus. About 1.0 mL sample was dissolved in 500 mL of aquadest at 37°C ± 0.5°C. Low stirring was performed by rotating the paddle at 60 rpm, and samples were assessed visually based on the emulsion rate of emulsification and final appearance [31].

2.7 | In Vitro Anticancer Activity

About 10 mg of optimized formula (F5) was weighed and dissolved in 100 µL DMSO. After that, dilution was carried out using culture medium to make test solutions with several concentrations of 12.5, 25, 50, 100, and 200 µg/mL. Both cells (HeLa and T47D) with a concentration of 1×10^4 cells/µL were distributed into wells (96-well plates) as much as 100 µL in each well and incubated for 24 h in a 5% CO₂ incubator. After incubation, the remaining media in the plate were removed and 100 µL of the test extract solution was added to each well. Next, it was incubated again in a 5% CO₂ incubator for 24 h. As a positive control, only

culture medium containing cancer cells was used without the addition of the test solution. As an anticancer comparison standard, doxorubicin was used with a concentration series of 100, 50, 25, 12.5, and 6.25 µg/mL. At the end of incubation, the culture medium was removed from the wells. In each well, 100 µL of culture medium and 10 µL of MTT solution (5 mg/mL) were added. Cells were incubated again for 4 h in a 5% CO₂ incubator. Living cells will react with MTT to form formazan crystals (dark purple blue color). The MTT reaction was stopped with 100 µL of SDS solution (10% in 0.01 N HCl), then the plate was shaken on a shaker for 10 min, and then incubated at room temperature in the dark overnight. Next, the absorbance of each well was read with an ELISA reader at a wavelength of 595 nm. IC₅₀ was calculated by plotting the percentage of cell death and concentration using linear regression equation [32]. The percentage of cell death was calculated as follows:

$$\% \text{ Cell death} = \frac{\text{Abs}(\text{cells control}) - \text{Abs}(\text{treated cells})}{\text{Abs}(\text{cells control})} \times 100 \% \quad (1)$$

2.8 | Determination of Total Phenolics

About 1 mL of the optimized formula (F5) was taken, and 0.4 mL of Folin-Ciocalteu reagent was added. Then, shake until homogeneous and allow to stand for 4–8 min. After that, 4 mL of 7% Na₂CO₃ solution was added and shaken until homogeneous, and aquabidest was added until the volume of 10 mL. After that, it was left for 2 h at room temperature. Next, the absorbance of each sample was measured. The experiment was performed in triplicate [33].

2.9 | Determination of Total Flavonoids

The optimized formula (F5) was taken as much as 1 mL and then mixed with 0.2 mL 10% AlCl₃, 0.2 mL 1 M potassium acetate, and 5.6 mL aquabidest. The mixture was incubated for 30 min at room temperature. After that, the absorbance of sample was measured using a UV-Vis spectrophotometer. The experiment was performed in triplicate [33].

2.10 | Determination of Total Saponins

About 25 µL of the optimized formula (F5) was taken (also pipetted ethanol as a blank) and mixed with 250 µL vanillin (8 g/

TABLE 2 | Summary of SNEDDS optimization formula and respond from D-optimal mixture design.

Formula	Component			Respond		
	VCO (%)	Tween 80 (%)	PG (%)	Particle size (nm)	Emulsification time (s)	Transmittance (%)
1	12	70	13	13.83	32	91.83
2	12	65.5	17.5	14.36	29	92.25
3	22	61	12	16.86	33	24.49
4	18.83	62.33	13.83	16.56	41	67.29
5	13	60	22	17.30	25	85.11
6	14.33	61.83	18.83	16.53	36	86.69
7	17.5	60	17.5	15.56	34	90.99
8	17.5	65.5	12	19.53	38	91.20
9	13.83	66.83	14.33	20.23	37	91.20

100 mL ethanol). Then, 2.5 mL of sulfuric acid (72%) was added. After that, the mixture was heated at 60°C for 15 min and left with cold water for 5 min. Then, the absorbance of sample was measured using a UV-Vis spectrophotometer. The experiment was performed in triplicate [33].

2.11 | Data Analysis

All experiments were conducted in triplicate, and the results are expressed as mean $\pm$ standard deviation. Statistical analysis of the SNEDDS formulation was performed using Design-Expert software. The appropriate mixture model (linear, quadratic, or special cubic) was selected based on sequential model sum of squares, lack-of-fit tests, and model significance ($p < 0.05$). Analysis of variance (ANOVA) was used to evaluate the significance of the model, individual components, and their interactions. Model adequacy was assessed using F values, p values, lack-of-fit tests, and adequate precision.

The cytotoxic activity and data visualizations were performed within the R software. The IC_{50} value was determined based on the drc (dose-response curve) package. Standard base R statistical functions were utilized to calculate linear regression equations ($y = bx + a$). Finally, all high-resolution dose-response plots and regression curves were generated using the ggplot2 package.

3 | Results and Discussion

3.1 | SNEDDS Optimization and Characterization

The formulation design in this study was guided by the balance between oil, surfactant, and cosurfactant to achieve optimal nanoemulsion characteristics [34, 35]. The selection of component ratios plays a crucial role in determining the efficiency of self-emulsification, droplet size, and overall system stability. A higher surfactant concentration reduces interfacial tension and facilitates the formation of smaller droplets, while the cosurfactant enhances interfacial flexibility and prevents phase separation [34].

The DMD enabled systematic evaluation of these interactions and their effects on key responses. This approach not only reduces the number of experimental runs but also provides a predictive model that can describe the relationship between formulation variables and SNEDDS performance. D-optimal design was selected due to its flexibility in handling constrained mixture systems and reducing experimental runs compared to central composite design (CCD) and Box-Behnken design (BBD), which are less suitable for mixture formulations where component proportions must sum to 100% [22, 23].

The concentration ranges used in the DMD of each component were derived from experimentally determined boundaries rather than arbitrary selection. Solubility and miscibility screening allowed the identification of a functional self-nanoemulsifying region capable of producing clear and physically stable nanoemulsions. The requirement for high surfactant content (60%–70% Tween 80) in this study is consistent with reports indicating that high-hydrophilic-lipophilic balance (HLB) surfactants are required to overcome the interfacial tension associated with natural extract solubilization and to promote spontaneous nanoemulsification. Meanwhile, limiting VCO and propylene

glycol to 12%–22% aligns with typical SNEDDS compositions and ensures adequate solubilization, physical stability, and safe excipient exposure [36–38].

As a respond, nine formulations were suggested and showed particle size of less than 100 nm (Table 2). The results also indicated that the ratio between the oil phase, surfactant, and cosurfactant must be balanced to produce small particle sizes with a low PDI. Tween 80, a surfactant characterized by a high HLB value, exhibits strong water-attracting properties that facilitate droplet formation and create an expansive surface area, enabling rapid dispersion of formulations in aqueous environments. Furthermore, surfactants play a crucial role in reducing surface free energy, thereby decreasing emulsion globule size when SNEDDS is mixed with water. Meanwhile, cosurfactants enhance the solubilization of active ingredients within the system and alleviate interfacial bending stress. They also support the formation of a flexible interfacial film [29]. Smaller particle sizes of nanoemulsions result in a larger surface area, leading to faster drug absorption. The combination of surfactants and cosurfactants also improves permeability by disrupting cell membrane lipids, thereby increasing drug absorption in the body.

The selection of the appropriate mixture model for each response was based on statistical evaluation, including sequential model sum of squares, lack-of-fit tests, and significance levels. The results showed that emulsification time and transmittance were best described by significant models ($p < 0.05$), while the particle size response exhibited a nonsignificant model F5 ($p > 0.05$) (Table 3).

The nonsignificant particle size model indicates that variations in component proportions within the studied range did not significantly influence droplet size. However, all formulations consistently produced nano-sized droplets (< 100 nm), confirming that the system remained within the acceptable SNEDDS range [39]. Therefore, particle size was retained in the optimization as a practical quality constraint, not as a predictive response variable, ensuring the final formulation meets nanometric requirements.

The characterization of SNEDDS formulations focused on critical quality attributes relevant to nanoemulsion performance. Particle size and PDI were evaluated to assess droplet size distribution and homogeneity, which are essential for stability and drug absorption. Emulsification time was measured to evaluate the efficiency of self-emulsification under mild agitation conditions, simulating gastrointestinal motility. Meanwhile, percentage of transmittance was used as an indirect indicator of nanoemulsion clarity and droplet size, where higher transmittance values correspond to smaller droplet sizes and better dispersion. These parameters are widely accepted as primary indicators for evaluating SNEDDS performance in early-stage formulation studies [39].

The selected characterization parameters collectively provide a comprehensive evaluation of SNEDDS performance. Particle size and PDI reflect the physical stability and uniformity of the system, while emulsification time and transmittance indicate the efficiency of nanoemulsion formation [39]. The combination of these parameters ensures that the optimized formulation meets the essential criteria for an effective SNEDDS system.

TABLE 3 | Statistical analysis of SNEDDS parameters.

ANOVA parameters	Particle size (nm) (Y ₁)	Emulsification time (s) (Y ₂)	Transmittance (%) (Y ₃)
Model ($p < 0.05$)	0.7928 (not significant)	0.0464 (significant)	0.0004 (significant)
R-squared	0.0745	0.9843	0.9999
Adj R-squared	0.2340	0.9371	0.9995
Adequate precision	1.2351	14.1802	154.6656
Mixture model	Linear mixture	Special cubic mixture	Special cubic mixture

Further ANOVA results (Table 4) revealed distinct effects of formulation variables on the evaluated responses. For particle size, the model was found to be not statistically significant ($F = 0.2415$, $p = 0.7928$), indicating that variations in the proportions of oil, surfactant, and cosurfactant did not significantly influence droplet size within the studied formulation range. This suggests that all tested formulations consistently produced nano-sized droplets, confirming the robustness of the SNEDDS system in maintaining particle size within the desired nanometer range [40]. Therefore, particle size was considered a noncritical but controlled parameter during optimization.

In contrast, emulsification time showed a statistically significant model ($F = 20.88$, $p = 0.0464$), indicating that formulation variables significantly affected the self-emulsification efficiency. The linear mixture component was significant ($p = 0.0365$), suggesting that the individual proportions of components strongly influence emulsification behavior [23]. Although interaction terms (AB, AC, and ABC) exhibited relatively high F values, their p values were slightly above 0.05, indicating a moderate but not statistically significant interaction effect. This implies that emulsification performance is primarily governed by the individual contribution of each component rather than their synergistic interactions.

For transmittance, the model was found to be highly significant ($F = 2694.42$, $p = 0.0004$), demonstrating a strong dependence of

nanoemulsion clarity on formulation composition [39, 41]. Both linear mixture and interaction terms (AB, AC, BC, and ABC) were statistically significant ($p < 0.05$), indicating that not only individual components but also their interactions play a critical role in determining the transparency of the system. The high F values observed for these terms suggest that transmittance is highly sensitive to formulation changes, making it a key parameter for optimization.

Overall, these results indicate that while particle size remained relatively unaffected by formulation variations, emulsification time and transmittance were the most critical responses influenced by component proportions. The significant effect on transmittance, in particular, highlights the importance of achieving an optimal balance between oil, surfactant, and cosurfactant to ensure efficient nanoemulsion formation and clarity [39]. These findings support the reliability of the DMD in identifying the optimal SNEDDS formulation with desirable physicochemical characteristics [23].

The SNEDDS characterization results (Table 5) show that all formulations produce nano-sized particles and exhibited a transparent appearance (Figure 1). The PDI values ranged from 0.17 ± 0.03 to 0.4 ± 6.79 , indicating that the system has a homogeneous particle size distribution and remains physically stable over time [35]. The transmission percentage of all formulations (except Formula 3) was close to 100%, indicating that

TABLE 4 | ANOVA summary for SNEDDS optimization responses.

Response	Source	Sum of squares	Regression coefficients	df	Mean square	F value	p value	Remark
Particle size	Model	2.68		2	1.34	0.2415	0.7928	Not significant
	Linear mixture	2.68		2	1.34	0.2415	0.7928	
	Residual	33.34		6	5.56			
Emulsification time	Model	185.92		6	30.99	20.88	0.0464	Significant
	Linear mixture	78.28		2	39.14	26.37	0.0365	
	AB	15.88	25.55	1	15.88	10.7	0.0821	
	AC	24.67	31.85	1	24.67	16.62	0.0552	
	BC	1.69	8.33	1	1.69	1.14	0.3982	
	ABC	19.84	176.7	1	19.84	13.37	0.0673	
	Residual	2.97		2	1.48			
Transmittance	Model	3974.83		6	662.47	2694.42	0.0004	Significant
	Linear mixture	2425.57		2	1212.78	4932.66	0.0002	
	AB	809.73	182.48	1	809.73	3293.37	0.0003	
	AC	1102.5	212.93	1	1102.5	4484.1	0.0002	
	BC	40.57	40.84	1	40.57	164.99	0.006	
	ABC	214.41	−580.9	1	214.41	872.04	0.0011	
	Residual	0.4917		2	0.2459			

TABLE 5 | Characterization of SNEDDS.

Formula	Particle size (nm)	PDI	Emulsification time (sec)	% transmittance
1	13.7 ± 0.41	0.21 ± 0.04	32 ± 2.00	91.20 ± 0.21
2	14.3 ± 0.35	0.13 ± 0.03	29 ± 2.08	90.99 ± 0.21
3	16.8 ± 0.45	0.17 ± 0.03	33 ± 2.08	24.48 ± 0.15
4	16.5 ± 0.05	0.23 ± 0.01	41 ± 1.52	67.29 ± 0.31
5	17.3 ± 0.51	0.17 ± 0.02	25 ± 1.00	85.11 ± 0.39
6	16.5 ± 0.11	0.21 ± 0.06	36 ± 2.00	86.69 ± 0.40
7	15.5 ± 0.11	0.16 ± 0.02	34 ± 2.00	90.99 ± 0.21
8	19.5 ± 0.25	0.40 ± 0.004	38 ± 2.00	91.20 ± 0.42
9	20.2 ± 0.20	0.38 ± 0.01	37 ± 1.00	91.20 ± 0.21

Note: Data represent mean ± SD, *n* = 3.

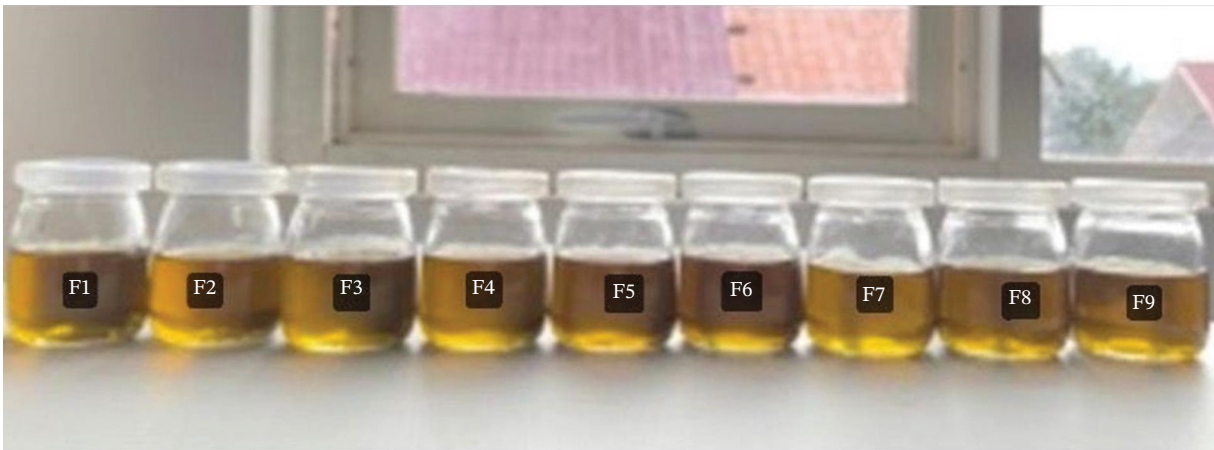


FIGURE 1 | Visual appearance of F1–F9 formulas of SNEDDS.

the droplet size was within the nanometer range. Emulsion droplet size plays a critical role in self-emulsification performance, as it affects the rate and extent of drug release and absorption [36]. Meanwhile, the low transmittance of Formula 3 (24.48%) indicates larger droplet size or partial emulsification and possible incomplete dispersion. This reflects suboptimal surfactant/cosurfactant ratios in certain formulations and supports the importance of optimization using D-optimal design.

To assess the ability of SNEDDS to form stable and transparent nanoemulsions after dispersion in gastrointestinal fluids, an emulsification time test was conducted. A good emulsification time is less than 1–2 min. Exceeding 2 min usually causes the emulsion system to become cloudy, which is not recommended for SNEDDS formulations. The results showed that all formulations exhibited emulsification times of less than 1 min [21].

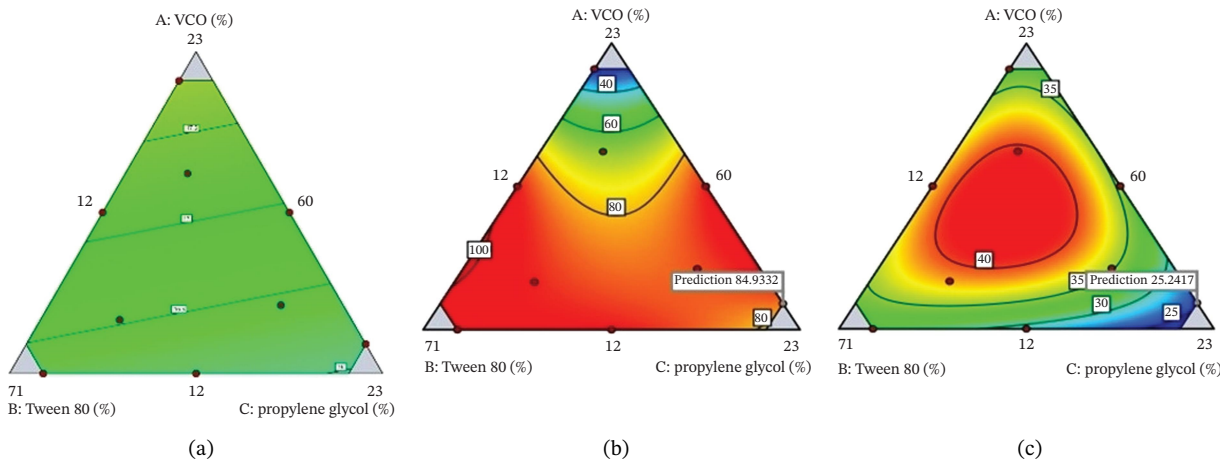


FIGURE 2 | Three-dimensional response plot on (a) particle size, (b) emulsification time, and (c) transmittance.

TABLE 6 | Respond criteria and importance.

Parameters	Goal	Lower limit	Upper limit	Importance
VCO	In range	12	22	3
Tween 80	Minimize	60	70	3
Propylene glycol	In range	12	22	3
Particle size	Minimize	13.70	20.20	3
Emulsification time	Minimize	25	41	3
% transmittance	Maximize	24.49	92.25	3

For SNEDDS optimization, the response analysis was illustrated using contour plot models (Figure 2). The particle size response contour plot showed that the material components had no significant effect on the particle size response, though the results remained within the acceptable nanometer range. The contour plot for emulsification time indicated that the blue regions represented the lowest emulsification times, while the red regions corresponded to the highest times. The blue area suggested that propylene glycol concentration influenced emulsification time. In the transmittance response diagram, gradients of green, yellow, and red were observed, where red areas indicated that Tween 80 and propylene glycol concentrations increased transmittance, while blue areas suggested that VCO reduced transmittance.

The components optimized in the SNEDDS formulation were VCO, Tween 80, and propylene glycol, each assigned an important level of 3. Higher importance value corresponds to a greater priority (Table 6) [37]. VCO and propylene glycol were optimized with a target value of 3, as these components fall within the SNEDDS requirements for nanoemulsion formation. Tween 80 was optimized with a minimized target (importance value 3) to achieve a lower surfactant concentration. The particle size response was also optimized with a minimize target (importance value 3), ensuring the final SNEDDS formulation achieved a nanometer size. The transmittance response was optimized with a maximized target (importance value 3) to achieve the highest transmittance. The emulsification time response, with an important value of 3, was optimized with a minimized target to ensure the fastest emulsification time. The predicted and experimental values of the formulations were closely aligned, with minimal differences (Table 7). The bias percentages were 7.32% for particle size, 0.9% for emulsification time, and 0.2% for transmittance, all below the 10% threshold, confirming the reliability of the optimal formula [38].

ANOVA analysis using Design-Expert software was performed to identify the optimal formula based on a desirability value close to 1 (Table 8). The first solution suggested by the DMD was selected as the optimal formula. This formula had the highest desirability value (0.862), comprising 13% VCO, 60% Tween 80, and 22% propylene glycol, corresponding to Formula 5. It was predicted to yield a particle size of 16.12 nm, an emulsification time of 25.24 s, and a transmittance of 84.93%. Nanoemulsions with high oil content tend to be unstable or prone to phase separation due to insufficient surfactant concentration to reduce the interfacial tension between the oil and water layers. The addition of co-surfactants reduces particle size by dissolving larger amounts of hydrophilic surfactants or hydrophobic drugs [38]. A high surfactant concentration can also reduce the interfacial tension by

TABLE 7 | Measurement results comparing DMD predictions with experiment.

Response	Prediction	Experiment	% bias
Particle size	16.12 nm	17.30 nm	7.32%
Emulsification time	25.24 s	25.00 s	0.90%
% transmittance	84.93%	85.11%	0.20%

surrounding oil droplets during emulsification, leading to nanometer-sized droplets [39].

Overall, the integration of rational component selection and statistical optimization strengthens the reliability of the developed SNEDDS formulation and supports its potential application for enhancing the delivery of hydrophobic herbal compounds [34, 35]. The results demonstrate that the balance between oil, surfactant, and cosurfactant plays a crucial role in determining SNEDDS performance. The use of DMD enabled efficient identification of the optimal formulation with minimal experimental runs, while maintaining desirable physicochemical characteristics. The close agreement between predicted and experimental values further confirms the reliability and predictive capability of the statistical model [23, 34].

However, this study has several limitations. First, zeta potential, cloud point, and robustness to dilution were not evaluated, which may provide additional insights into the physicochemical stability of the SNEDDS system. Second, no pseudo-ternary phase diagram was constructed, as the formulation space was explored using a DMD approach. Third, comprehensive stability studies under different storage conditions were not conducted due to the scope of this work being limited to formulation optimization. Therefore, future studies should focus on extended physicochemical characterization, stability evaluation, and in vivo performance to further validate the applicability of the developed SNEDDS system.

3.2 | In Vitro Anticancer Assay and Phytochemical Compounds of Optimized SNEDDS Formula

The anticancer assay was in vitro tested on HeLa and T47D cell lines. Both cells were selected as they represent two of the most common hormone-related cancers in women: cervical cancer and breast cancer, respectively. HeLa cells are widely used as a robust model for screening cytotoxic activity due to their aggressive proliferation and high sensitivity to chemotherapeutic compounds [42]. T47D cells provide a representative model of estrogen receptor-positive breast cancer, enabling evaluation of the potential effect on hormone-dependent tumors [43].

TABLE 8 | D-optimal mixture design for optimum formula.

VCO	Tween 80	PG	Particle size (nm)	Emulsification time (s)	Transmittance (%)	Desirability
13	60	22	16.12	25.24	84.93	0.862
12.5	60.5	22	16.04	24.68	81.13	0.844
19.5	63.5	12	17.44	36.77	68.79	0.468
17.5	65.5	12	17.14	37.78	91.34	0.452

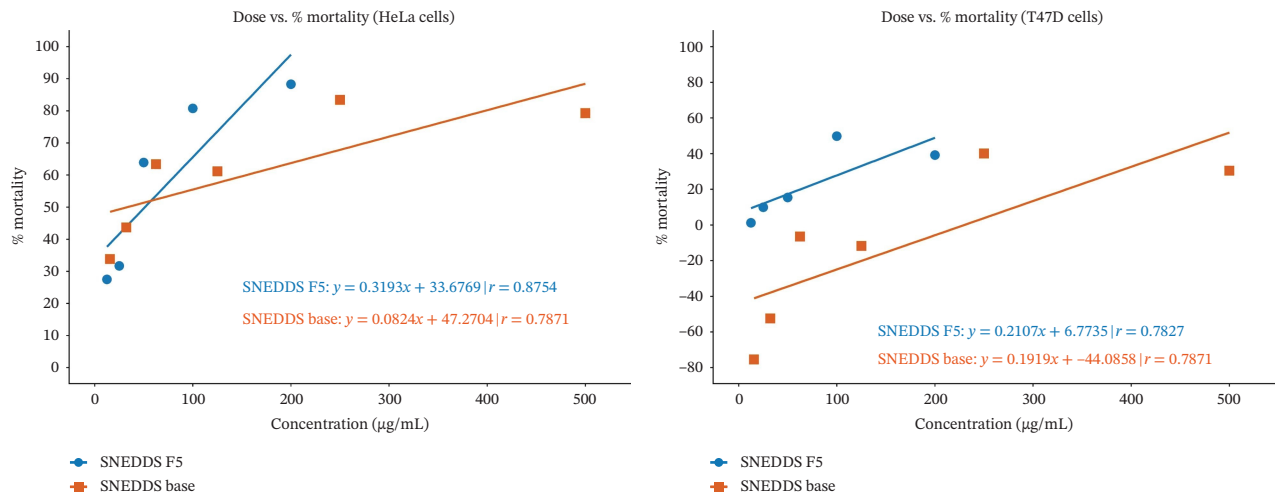


FIGURE 3 | Cytotoxic activity of the optimal SNEDDS formula (F5) and SNEDDS base against HeLa (left) and T47D cell lines (right).

TABLE 9 | Total phenolics, total flavonoids, and total saponins of optimal formula (F5).

Formula	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Total saponins (mg EE/g)
5	54.90 ± 0.77	43.85 ± 2.18	271.27 ± 7.10

Together, they provide complementary evidence for anticancer activity across two distinct cancer types.

The cytotoxicity of the optimized SNEDDS formula (F5) was stronger against HeLa cells ($IC_{50} = 21.8 \mu\text{g/mL}$) compared to T47D cells ($IC_{50} = 117.3 \mu\text{g/mL}$) (Figure 3). This difference may be attributed to the intrinsic biological characteristics of the cell lines. HeLa cells are highly proliferative and generally more sensitive to compounds that interfere with DNA replication and cell division [44]. In contrast, T47D cells, being estrogen receptor-positive breast cancer cells, often require specific hormonal or receptor-mediated pathways to exhibit stronger responses [43]. This could partially explain the reduced sensitivity observed. These findings suggest that the SNEDDS formulation might be more suitable for targeting fast-proliferating, hormone-independent cancers. Nonetheless, broader validation in additional cancer and noncancer cell lines is necessary to fully interpret these differences [45].

The optimized SNEDDS formula (F5) also exhibited significantly improved anticancer activity compared with the SNEDDS base, as demonstrated by markedly lower IC_{50} values in both HeLa and T47D cancer cell lines (Figure 3). In HeLa cells, F5 achieved approximately two-fold lower than the SNEDDS base formula

($41.6 \mu\text{g/mL}$), indicating enhanced cytotoxic potency. In T47D cells, the SNEDDS base showed limited activity ($IC_{50} = 587.4 \mu\text{g/mL}$), and it was significantly different with F5 ($p < 0.05$), reflecting a pronounced enhancement in anticancer efficiency. This improvement is consistent with previous reports showing that SNEDDS can increase the solubility, stability, and membrane permeability of phytochemical-based compounds, thereby enhancing their biological activity in vitro and in vivo [46]. The likely mechanism involves increased solubilization and nano-sized droplets facilitating enhanced uptake of hydrophobic bioactive compounds into cancer cells, overcoming limitations associated with poor water solubility and bioavailability [46].

The determination of phytochemical compounds concentrations in the optimized SNEDDS formula revealed that the optimized formula (F5) contained higher total saponins ($271.27 \pm 7.10 \text{ mg EE/g}$) compared to total phenolics ($54.90 \pm 0.77 \text{ mg GAE/g}$) and total flavonoids ($43.85 \pm 2.18 \text{ mg QE/g}$) (Table 9). This result was supported by previous research that saponins from *B. medicinalis*, which are triterpenoid and steroid glycosides, had been reported to exhibit anticancer activity [3, 27].

Finally, the inclusion of HeLa and T47D cell lines strengthens the reliability of our findings, as both are well-established,

authenticated cancer models. However, because this study is limited to two human cancer cell lines, the conclusions regarding anticancer efficacy remain preliminary. Additional evaluations in other cancer models and in vivo studies are required before definitive translational claims can be made.

4 | Conclusion

The DMD was employed as an optimization tool for the SNEDDS formulation. The optimized SNEDDS formula (F5), combining the ethyl acetate fraction of *B. medicinalis* and the ethanol fraction of *M. oleifera*, consisted of 13% VCO, 60% Tween 80, and 22% propylene glycol. This optimal formulation exhibited good SNEDDS characteristics including particle size of 17.3 ± 0.51 nm, a PDI of 0.17 ± 0.02 , an emulsification time of 25 ± 1.00 s, and percent transmittance of $85.11 \pm 0.39\%$ with potent cytotoxic activity against HeLa and T47D cancer cells. It also has high levels of saponin, phenolic, and flavonoid compounds.

Acknowledgments

The authors acknowledge Indonesia Endowment Fund for Education Agency (LPDP) and National Research and Innovation Agency (BRIN), Republic of Indonesia, for the financial support through Riset Inovasi Untuk Indonesia Maju (RIIM) 2023 grants with contract numbers 186/IV/KS/11/2023 and 3310/UN28.16/AL.04/2023. During the preparation of this work, the authors used ChatGPT AI in order to improve the sentences. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Funding

This study was supported by National Research and Innovation Agency (BRIN), 186/IV/KS/11/2023, and Indonesia Endowment Fund for Education Agency (LPDP), 3310/UN28.16/AL.04/2023.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. M. S. Zubair, S. Anam, A. Khumaidi, Y. Susanto, M. Hidayat, and A. Ridhay, "Molecular Docking Approach to Identify Potential Anti-cancer Compounds From Begonia (*Begonia* sp.)," *AIP Conference Proceedings* 1741 (2016): 080005, <https://doi.org/10.1063/1.4958513>.
2. S. Anam, A. Ritna, F. Dwimurti, D. Rismayanti, and M. S. Zubair, "Aktivitas Sitotoksik Ekstrak Metanol Benalu Batu (*Begonia* sp.): Ethnomedicine Suku Wana Sulawesi Tengah," *Jurnal Ilmu Kefarmasian Indonesia* 12, no. 1 (2014): 10–16.
3. M. S. Zubair, W. M. Alarif, M. A. Ghandourah, S. Anam, and I. Jantan, "Cytotoxic Activity of 2-O- β -Glucopyranosil Cucurbitacin D From Benalu Batu (*Begonia* sp.) Growing in Morowali, Central Sulawesi," *Indonesian Journal of Chemistry* 20, no. 4 (June 2020): 766, <https://doi.org/10.22146/ijc.43626>.
4. A. Khumaidi, A. Widodo, A. W. Nugrahani, E. Sasmito, and N. Fakhruddin, "Profil Proliferasi Sel Limfosit Benalu Batu (*Begonia medicinalis*) Asal Kabupaten Morowali Utara Provinsi Sulawesi Tengah," *Jurnal Ilmu Kefarmasian Indonesia* 18, no. 1 (2020): 61–67.

5. M. S. Zubair, S. Q. Khairunisa, E. Sulastri, et al., "Antioxidant and Antiviral Potency of *Begonia medicinalis* Fractions," *Journal of Basic and Clinical Physiology and Pharmacology* 32, no. 4 (June 2021): 845–851, <https://doi.org/10.1515/jbcpp-2020-0476>.
6. A. Benarima, S. E. Laouini, B. B. Seghir, Y. Belaiche, and M. R. Ouahrani, "Optimization of Ultrasonic-Assisted Extraction of Phenolic Compounds From *Moringa oleifera* Leaves Using Response Surface Methodology," *Asian Journal of Research in Chemistry* 13, no. 5 (2020): 307–311, <https://doi.org/10.5958/0974-4150.2020.00059.0>.
7. A. Djemoui, D. Djemoui, L. Souli, A. Souadia, and M. Gouamid, "The Antidiabetic, Antioxidant Properties In Vitro of *Moringa oleifera* Flowers Extracts Grown in Sahara of Algeria," *AJRC* 7 (June 2021): 173–178, <https://doi.org/10.52711/0974-4150.2021.00032>.
8. M. Kumbhare, T. Sivakumar, and A. Surana, "Evaluation of Hypoglycemic Potential of *Moringa oleifera* Bark Extracts on Normal and Alloxanized Diabetic Rats," *RJPDT* 30 (April 2021): 95–99, <https://doi.org/10.52711/0975-4377.2021.00017>.
9. S. Mali, S. Bendre, and S. Patil, "Overview of Pharmacognostical and Pharmacological Properties of *Moringa oleifera*," *AJPT* 5 (March 2022): 77–83, <https://doi.org/10.52711/2231-5713.2022.00013>.
10. N. N. Sejal, "Moringa oleifera: A Review on Medicinal Properties and Their Commercial Applications," *Asian Journal of Plant Science* 27 (May 2024): 185–188, <https://doi.org/10.52711/2231-5659.2024.00030>.
11. E. Sulastri, M. S. Zubair, N. I. Anas, et al., "Total Phenolic, Total Flavonoid, Quercetin Content and Antioxidant Activity of Standardized Extract of *Moringa oleifera* Leaf From Regions With Different Elevation," *Pharmacognosy Journal* 10, no. 6 (November 2018): s104–s108, <https://doi.org/10.5530/pj.2018.6s.20>.
12. T. L. C. Oldoni, N. Merlin, M. Karling, et al., "Bioguided Extraction of Phenolic Compounds and UHPLC-ESI-Q-TOF-MS/MS Characterization of Extracts of *Moringa oleifera* Leaves Collected in Brazil," *Food Research International* 125 (November 2019): 108647, <https://doi.org/10.1016/j.foodres.2019.108647>.
13. M. S. Zubair, A. Syamsidi, E. Sulastri, et al., "Immunomodulatory Effects of Combined Ethanolic Extracts of *Begonia medicinalis* and *Moringa oleifera* in Wistar Rats Infected With *Staphylococcus aureus*," *MJCHEM* 24, no. 4 (December 2022): <https://doi.org/10.55373/mjchem.v24i4.144>.
14. J. Baloch, M. F. Sohail, H. S. Sarwar, et al., "Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Improved Oral Bioavailability of Chlorpromazine: In Vitro and In Vivo Evaluation," *Medicina* 55, no. 5 (May 2019): 210, <https://doi.org/10.3390/medicina55050210>.
15. J. Dolai, K. Mandal, and N. R. Jana, "Nanoparticle Size Effects in Biomedical Applications," *ACS Applied Nano Materials* 4, no. 7 (July 2021): 6471–6496, <https://doi.org/10.1021/acsanm.1c00987>.
16. M. Taiyeb, H. Hartati, A. Arwansyah, et al., "Self-Nanoemulsifying Drug Delivery System (SNEDDS) Formulation and Molecular Docking of Mahogany Seed Extract (*Swietenia mahagoni*) as Anti-Hyperglycemic," *Informatics in Medicine Unlocked* 47 (2024): 101517, <https://doi.org/10.1016/j.imu.2024.101517>.
17. N. Zhang, F. Zhang, S. Xu, K. Yun, W. Wu, and W. Pan, "Formulation and Evaluation of Luteolin Supersaturable Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) for Enhanced Oral Bioavailability," *Journal of Drug Delivery Science and Technology* 58 (August 2020): 101783, <https://doi.org/10.1016/j.jddst.2020.101783>.
18. A. B. Buya, A. Beloqui, P. B. Memvanga, and V. Pr  at, "Self-Nano-Emulsifying Drug-Delivery Systems: From the Development to the Current Applications and Challenges in Oral Drug Delivery," *Pharmaceutics* 12, no. 12 (December 2020): 1194, <https://doi.org/10.3390/pharmaceutics12121194>.
19. S. Inugala, B. B. Eedara, S. Sunkavalli, et al., "Solid Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) of Darunavir for Improved Dissolution and Oral Bioavailability: In Vitro and In Vivo

- Evaluation," *European Journal of Pharmaceutical Sciences* 74 (July 2015): 1–10, <https://doi.org/10.1016/j.ejps.2015.03.024>.
20. R. Nazari-Vanani, L. Moezi, and H. Heli, "In Vivo Evaluation of a Self-Nanoemulsifying Drug Delivery System for Curcumin," *Bio-medicine & Pharmacotherapy* 88 (April 2017): 715–720, <https://doi.org/10.1016/j.biopha.2017.01.102>.
 21. F. P. Schmied, A. Bernhardt, A. Engel, and S. Klein, "A Customized Screening Tool Approach for the Development of a Self-Nanoemulsifying Drug Delivery System (SNEDDS)," *AAPS PharmSciTech* 23, no. 1 (January 2022): 39, <https://doi.org/10.1208/s12249-021-02176-7>.
 22. D. C. Montgomery, *Design and Analysis of Experiments*, 8th ed. (John Wiley & Sons, Inc, 2013).
 23. R. H. Myers, D. C. Montgomery, and C. M. Anderson-Cook, *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*, 4th ed. (Wiley series in Probability and Statistics, 2016).
 24. N. Asita, M. S. Zubair, Y. Syukri, and E. Sulastri, "Formulation of Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Combined 70% Ethanolic of Begonia medicinalis Herbs and Moringa oleifera Leaves," *Pharmacy Education* 24, no. 3 (May 2024): 304–309, <https://doi.org/10.46542/pe.2024.243.304309>.
 25. M. Zubair, J. Poluan, F. Hayati, A. Ramadani, E. Sulastri, and Ihwan, "Self-Nanoemulsifying Drug Delivery System (SNEDDS) Prepared From Begonia medicinalis and Moringa oleifera Ethanol Extracts," *Tropical Journal of Pharmaceutical Research* 24, no. 1 (February 2025): 1–8, <https://doi.org/10.4314/tjpr.v24i1.1>.
 26. M. S. Zubair, S. E. Jusriani, and A. Syamsidi, "Arwansyah. Optimization of Self Nano-Emulsifying Drug Delivery System (SNEDDS) Formula of Combined 70% Ethanolic Extract of Benalu Batu (Begonia medicinalis) Herbs and Kelor (Moringa oleifera L.) Leaves Using Simplex Lattice Design Method," *International Journal of Applied Pharmaceutics* 7 (January 2025): 432–438, <https://doi.org/10.22159/ijap.2025v17i1.52165>.
 27. M. S. Zubair, W. M. Alarif, M. A. Ghandourah, and S. Anam, "A New Steroid Glycoside From *Begonia* sp.: Cytotoxic Activity and Docking Studies," *Natural Product Research* 35, no. 13 (July 2021): 2224–2231, <https://doi.org/10.1080/14786419.2019.1669026>.
 28. M. Kaszuba, D. McKnight, M. T. Connah, F. K. McNeil-Watson, and U. Nobbmann, "Measuring Sub Nanometre Sizes Using Dynamic Light Scattering," *Journal of Nanoparticle Research* 10, no. 5 (May 2008): 823–829, <https://doi.org/10.1007/s11051-007-9317-4>.
 29. M. Danaei, M. Dehghankhold, S. Ataei, et al., "Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nano-carrier Systems," *Pharmaceutics* 10, no. 2 (May 2018): 57, <https://doi.org/10.3390/pharmaceutics10020057>.
 30. A. Nasr, A. Gardouh, and M. Ghorab, "Novel Solid Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) for Oral Delivery of Olmesartan Medoxomil: Design, Formulation, Pharmacokinetic and Bioavailability Evaluation," *Pharmaceutics* 8, no. 3 (June 2016): 20, <https://doi.org/10.3390/pharmaceutics8030020>.
 31. USP, *U.S. Pharmacopoeia-National Formulary [USP 38 NF 33]* (United States Pharmacopeial Convention, 2015).
 32. M. S. Zubair, S. Anam, and S. Lallo, "Cytotoxic Activity and Phytochemical Standardization of Lunasia amara Blanco Wood Extract," *Asian Pacific Journal of Tropical Biomedicine* 6, no. 11 (November 2016): 962–966, <https://doi.org/10.1016/j.apjtb.2016.04.014>.
 33. M. S. Zubair, A. Syamsidi, S. E. Ihwan, et al., "Immunomodulatory Activity of Begonia medicinalis Ethanolic Extract in Experimental Animals," *Indonesian Journal of Pharmacy* (December 2022): <https://doi.org/10.22146/ijp.3588>.
 34. S. Priani, A. Nurhaliza, A. Chaerunisaa, G. Wilar, and I. Sopyan, "Solidification of SNEDDS Using Mesoporous Carriers (2020–2025): A Review of Design, Biopharmaceutical Enhancement, and Therapeutic Impact," *Drug Design, Development and Therapy* 19 (December 2025): 11989–12017, <https://doi.org/10.2147/DDDT.S577016>.
 35. S. E. Priani, A. Nurhaliza, R. Aryani, G. Wilar, A. Y. Chaerunisaa, and I. Sopyan, "Recent Progress in Supersaturation-Based SNEDDS: Formulation, Mechanism, and Biopharmaceutical Performance," *Open* 25 (September 2025): 100252, <https://doi.org/10.1016/j.onano.2025.100252>.
 36. S. S. El-Mancy, A. E. El-Haddad, W. A. Alshareef, A. M. Saadeldeen, S. Z. El-Emam, and O. S. Elnahas, "Enhancement of Antimicrobial and Antiproliferative Activities of Standardized Frankincense Extract Using Optimized Self-Nanoemulsifying Delivery System," *Scientia Pharmaceutica* 89, no. 3 (August 2021): 36, <https://doi.org/10.3390/scipharm89030036>.
 37. S. M. Fayez, O. S. Elnahas, A. M. Fayez, and S. S. El-Mancy, "Coconut Oil Based Self-Nano Emulsifying Delivery Systems Mitigate Ulcerogenic NSAIDs Side Effect and Enhance Drug Dissolution: Formula Optimization, In-Vitro, and In-Vivo Assessments," *International Journal of Pharmaceutics* 634 (March 2023): 122666, <https://doi.org/10.1016/j.ijpharm.2023.122666>.
 38. A. J. Humberstone and W. N. Charman, "Lipid-Based Vehicles for the Oral Delivery of Poorly Water Soluble Drugs," *Advanced Drug Delivery Reviews* 25, no. 1 (April 1997): 103–128, [https://doi.org/10.1016/S0169-409X\(96\)00494-2](https://doi.org/10.1016/S0169-409X(96)00494-2).
 39. R. Ulfa and H. K. Pratiwi, "Review: Characterisation and Dissolution of Low Solubility Active Substances in S-SNEDDS Preparations With Various Manufacturing Methods," *Journal of Pharmaceutical and Clinical Research* 10, no. 2 (August 2025): 263, <https://doi.org/10.20961/jpscr.v10i2.65818>.
 40. C. W. Pouton and C. J. H. Porter, "Formulation of Lipid-Based Delivery Systems for Oral Administration: Materials, Methods and Strategies," *Advanced Drug Delivery Reviews* 60, no. 6 (March 2008): 625–637, <https://doi.org/10.1016/j.addr.2007.10.010>.
 41. M. D. Saeed, K. U. Shah, A. Nawaz, et al., "Fabrication and Evaluation of Solidified Nanoemulsion Designs for Systemic Delivery of Atorvastatin Through the Lung," *Scientific Reports* 15, no. 1 (July 2025): 20884, <https://doi.org/10.1038/s41598-025-05646-1>.
 42. J. R. Masters, "HeLa Cells 50 Years On: The Good, the Bad and the Ugly," *Nature Reviews Cancer* 2, no. 4 (April 2002): 315–319, <https://doi.org/10.1038/nrc775>.
 43. D. L. Holliday and V. Speirs, "Choosing the Right Cell Line for Breast Cancer Research," *Breast Cancer Research* 13, no. 4 (August 2011): 215, <https://doi.org/10.1186/bcr2889>.
 44. J. J. M. Landry, P. T. Pyl, T. Rausch, et al., "The Genomic and Transcriptomic Landscape of a HeLa Cell Line," *G3 Genes/Genomes/Genetics* 3, no. 8 (August 2013): 1213–1224, <https://doi.org/10.1534/g3.113.005777>.
 45. R. Kaur, A. Sharma, and N. Wijekoon, "Breast Cancer Preclinical Models: A Vital Resource for Comprehending Disease Mechanisms and Therapeutic Development," *EXCLI Journal* 24 (2025): Doc267–Doc285, <https://doi.org/10.17179/EXCLI2024-7973>.
 46. I. Julio, M. S. Zubair, and Y. Syukri, "Pharmacological Activity of Plant Extracts in Self-Nanoemulsifying Drug Delivery System Dosage Form: Narrative Review," *Jurnal Sains Farmasi & Klinis* 11, no. 1 (June 2024): 7–16, <https://doi.org/10.25077/jsfk.11.1.7-16.2024>.