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Design and optimization of *Eleutherine palmifolia* extract-loaded SNEDDS using HLB approach

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ABSTRACT: The novel surfactant system was designed to formulation a self-nanoemulsifying drug delivery system (SNEDDS) based on Hydrophilic Lipophilic Balance (HLB) value. This study aimed to prepare and characterize the *Eleutherine palmifolia* (*E. palmifolia*) extract-loaded self-nanoemulsifying drug delivery system (SNEDDS) using a Hydrophilic Lipophilic Balance (HLB) approach. *E. palmifolia* formulation was prepared using the Miglyol 812 as oil, with two hydrophilic surfactants (tween 20, tween 80) mixed with two lipophilic surfactants (Span 20 and Transcutol). The formulated four binary surfactant combinations with HLB ranging from 11 to 15, and co-surfactant polyethylene glycol [PEG] 400. The characteristics of the optimal formulation showed transmittance value of 97.97 ± 0.17%; emulsification time on artificial intestinal fluid (AIF) media of 52.00 ± 0.03 seconds; artificial gastric fluid (AGF) media of 50.00 ± 0.03 seconds; pH of 8.90 ± 0.06; viscosity of 32.81 ± 0.41 cps; and particle size of 12.08 nm. The selected formulation was a ratio of 2:7:1 composed of Miglyol 812, Tween 80, Transcutol, and PEG 400.

KEYWORDS: SNEEDS; self-nanoemulsifying; formulation; *Eleutherine palmifolia*; HLB approach.

1. INTRODUCTION

Eleutherine palmifolia also known as Dayak onion, is an indigenous plant of Kalimantan, Indonesia, used by the Dayak people to cure various diseases. Its phytochemical constituents, which function as anticancer, is naphthoquinone [1,2]. Aside from its bioactivity as an anticancer and antioxidant. It is a lipophilic compound with a logP value of 3.933, meaning that it has low solubility in water. Hence, there is a need to develop the self-nanoemulsifying drug delivery system (SNEDDS) to improve the bioavailability of naphthoquinone contained in *E. palmifolia* extract [3].

Thus, increased bioavailability translates to an increase in drug solubility, protection against enzymatic hydrolysis, and an increase in droplets specific surface area. In general, drug distribution in the gastrointestinal tract is quite large and an increase in permeability due to surfactant induction is critical in the process [4]. This technology of pharmaceutical preparation and delivery systems has the advantage of producing drugs which able to penetrate intercellular spaces [5]. Hence, nanoparticles as the latest drug delivery systems can increase drug movement to receptors [6]. In developing pharmaceutical technology-based drug delivery systems, it is essential to factor in the compounds ability to penetrate the tissue involved, which is the primary focus of SNEDDS [7].

One of the advantages of SNEDDS is the ability to present drugs in dissolved form in the lumen of the gastrointestinal tract (GI). Thus, providing a more extensive interface area for drug absorption [8]. SNEDDS involved an isotropic mixture of oils, surfactants, and co-surfactants with the capacity of forming spontaneous nanoemulsions when in contact with stomach fluids [9,10]. The advantage of nanoemulsion in water is its ability to carry lipophilic drugs in oil to emulsify in water and ultimately increase its solubility while in the body [11].

In this study, Mygliol 812 was selected as the oil phase. Mygliol 812 is a medium-chain triglyceride use for oral administration. It has a higher solvent capacity than long-chain triglycerides and tends not to be rancid due to oxidation [13]. The mixture of surfactant to obtain HLB range 11 to 15. Combinations of both surfactants were designed to have mixed HLB > 10 in order to form an O/W system, which is easy to become emulsion spontaneously in aqueous media. The optimum concentrations of oil, surfactant, and co-surfactant necessary

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to promote self emulsification are determined by constructing a ternary phase diagram. However, the experimental determination of phase diagram is a time consuming process, requiring careful formulation and characterization of all phases in a system the alternative method to prepare SNEDDS by calculating Hydrophilic Lipophilic Balance (HLB). Although the HLB system is not absolute predicting the formulation behavior, it is an excellent starting point for achieving emulsification.

The HLB of the surfactant offers essential information on its potential use in the formulation of SNEDDS. A mixture of different surfactants often exhibits synergism in their effects on the properties of a system. This synergism can be attributed to non-ideal mixing effects in the aggregates. This results in critical micellization concentration and interfacial tension that is substantially lower than expected based on the unmixed surfactants properties. Therefore, it is interesting to investigate the effect of the HLB of different blends of surfactants. Hence, the present study developed SNEDDS containing *E. palmifolia* extract, a SNEDDS system for natural products using the HLB approach. Afterward, the formulation was subsequently subjected to physicochemical characterization.

2. RESULTS

2.1. Surfactants Selection and SNEDDS Formulation

The screening was intended to discover the harmony of Miglyol 812 (medium-chain triglyceride), the surfactant and the co-surfactant. Both substance's synergy effect was accumulated to reduce the surface tension between oil and water to obtain clear and homogenous nanoemulsion.

In SNEDDS formulation, the HLB method can be used as a starting point to acquire excellent emulsification characteristics. More than one surfactant can be blended to get desirable HLB. Selected surfactants should have good miscibility with other SNEDDS formulation components to produce a stable and homogenous system. Another criterion is having relatively low toxicity for oral administration. HLB method was used to predict the HLB value of emulsion and to design a mixed ratio of two surfactants to yield a system with HLB of >10. The results are shown in Table 1.

Table 1. Result visually of surfactant selection and SNEDDS formulation.

HLB Value	Formulation	Visually	HLB Value	Formulation	Visually
11	F1	Coalescence	11	F21	Coalescence
12	F2	Coalescence	12	F22	Coalescence
13	F3	Coalescence	13	F23	Coalescence
14	F4	Coalescence	14	F24	Coalescence
15	F5	Coalescence	15	F25	Coalescence
11	F6	Coalescence	11	F26	Coalescence
12	F7	Coalescence	12	F27	Coalescence
13	F8	Coalescence	13	F28	Homogeneous
14	F9	Homogeneous	14	F29	Coalescence
15	F10	Coalescence	15	F30	Homogeneous
11	F11	Coalescence	11	F31	Coalescence
12	F12	Coalescence	12	F32	Coalescence
13	F13	Coalescence	13	F33	Coalescence
14	F14	Coalescence	14	F34	Coalescence
15	F15	Coalescence	15	F35	Homogeneous
11	F16	Homogeneous	11	F36	Homogeneous
12	F17	Homogeneous	12	F37	Coalescence
13	F18	Homogeneous	13	F38	Coalescence
14	F19	Homogeneous	14	F39	Coalescence
15	F20	Coalescence	15	F40	Homogeneous

The properties of surfactants, such as HLB value in oil, viscosity, and affinity for oil, strongly affect nano emulsification and droplets the size of nanoemulsion [9]. The mixture of hydrophilic and lipophilic surfactants can be used to form nanoemulsion with desired characteristics. HLB of Surfactant with < 10 is lipophilic and can produce W/O emulsion, whereas over >10 is hydrophilic and can produce O/W emulsion. Surfactant concentration plays a role in the formation of droplets size in nanometric [21]. The bigger the ratio between hydrophilic and lipophilic surfactants, the higher the HLB. The proper mixture of surfactants with lower and higher HLB may produce stable nanoemulsion even diluted with water. The proper mixture may also lower the interfacial tension to facilitate the dispersion process by forming a flexible film that can readily deform around droplets. Several forty (40) formulations of SNEDDS with HLB between 11-15 using different oil, surfactant, and co-surfactant (Table 3) ratios were prepared and evaluated for their stability after 24h of storage at 25°C ± 2°C. The most stable formulation, which did not show phase separation, was selected as a template for SNEDDS *E. palmifolia* extract formulation. Ten (10) formulations, which shows the absence of phase separation (homogeneous) were obtained after 24h of storage (Table 1). Higher HLB value showed higher hydrophilicity affected the reduction of curvature on the oil interface, thereby increasing the solubility and making the smaller droplets. Therefore, in this study the stable SNEDDS formulation with the highest HLB value was selected. Moreover, the best formulation was based on higher oil concentration to obtain a protective effect on protein drugs but producing nanoemulsion with small droplets [14]. The selection of surfactants with as low as possible concentration could reduce the risk of toxicity and irritation. Ten formulations were stable. They were selected as they had the highest HLB that made the emulsification process more comfortable, the highest concentration of oil component, and the lowest surfactant concentration. Further analysis was done for those ten formulations.

2.2. Characterization of *E. palmifolia* Extract-loaded SNEDDS

Based on the screening of surfactant selection results, ten formulation mixtures of oil, surfactants, and co-surfactants were devoid of phase separation, including F9, F16, F17, F18, F19, F28, F30, F35, F36, F36, and F40. The basic for selecting the optimal formulation is (1) having the highest HLB value and (2) the least surfactant. In addition, those with high HLB comprise F9 (HLB 14), F19 (HLB 14), F30 (HLB 15), F35 (HLB 15), and F40 (HLB 15). Furthermore, F30 and F40 did not continue to the next stage as surfactants were not combined in both formulations (70.00:0.00). Similarly, F9 was halted at a later stage due to the transmittance percentage of 68.29% and cloudy particles after emulsification in the media. Subsequently, the continued formulation following stability test was F19 and F35, due to the high HLB value and inherent appropriate characteristics.

Table 2. Result characterization of *E. palmifolia* extract-loaded SNEDDS.

HLB Value	Selected SNEDDS formulation	Percentage of transmittance (%) ± SD	Emulsification time (second)		pH ± SD	Viscosity (cps) ± SD	Droplet size (nm) ± SD
			AIF ± SD	AGF ± SD			
14	F9	68.29 ± 0.30	55.00 ± 0.26	32.00± 0.04	8.90 ± 0.06	37.73 ± 0.88	175.30 ± 1.43
11	F16	98.02 ± 0.33	10.00 ± 0.01	20.00 ± 0.02	7.20 ± 0.12	4.46 ± 0.42	6.16 ± 0.88
12	F17	98.29 ± 0.00	32.00 ± 0.02	23.00 ± 0.04	7.60 ± 0.06	13.54 ± 0.29	10.80 ± 1.22
13	F18	93.57 ± 0.22	41.00 ± 0.02	25.00 ± 0.03	7.30 ± 0.06	22.68 ± 0.31	12.73 ± 0.55
14	F19	97.97 ± 0.17	52.00 ± 0.03	50.00 ± 0.03	8.90 ± 0.06	32.81 ± 0.41	12.08 ± 0.69
13	F28	96.92 ± 0.61	28.00 ± 0.02	22.00 ± 0.03	9.10 ± 0.06	20.44 ± 0.67	15.73 ± 0.21
15	F30	98.09 ± 0.45	20.00 ± 0.02	22.00 ± 0.03	7.70 ± 0.17	33.22 ± 0.25	12.68 ± 0.27
15	F35	95.60 ± 0.86	41.00 ± 0.04	43.00 ± 0.03	9.10 ± 0.06	37.73 ± 0.88	10.10 ± 0.65
11	F36	97.60 ± 0.01	22.00 ± 0.02	12.00 ± 0.02	7.40 ± 0.06	40.46 ± 0.43	10.25 ± 0.33
14	F40	97.16 ± 0.66	40.00 ± 0.03	27.00 ± 0.02	7.60 ± 0.06	13.54 ± 0.29	11.05 ± 0.50

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

A surfactant dissolved in a liquid can either adsorb at the interface or self-assemble to form micelles, resulting from the lipophilic effect. The surfactant's hydrophilic group tends to be expulsed from the liquid in which the surfactant is dissolved. The adsorption of surfactants at the interface induces a structural change in the interfacial area and, in many cases, decreases the interfacial tension. It seems evident that, by changing the surfactant, the interfacial tension decreases to a different degree, which affects the final droplet size [28]. The HLB method has been demonstrated to be a useful tool in selecting the optimal type of surfactants for a specific oil phase [29]. SNEDDS was prepared with ratio 2:7:1 and 1:7:2. The mixed surfactants at HLB values ranging from 10 to 15. Four series of mixed surfactants, consisting of two different types of hydrophilic surfactants (i.e., Tween 80, Tween 20) and two types of lipophilic surfactants (i.e., Span 20 and Transcutol), were examined to determine the suitable HLB to obtain SNEDDS. Blends of surfactants at various ratios were used to prepare mixed surfactants with a range of HLB values (Table 4). The results show that, although the HLB value is the same, there are differences in the characteristics obtained, including droplet size. The size of the droplets produced depends on the molecular structure of the surfactant. In this study, nanoemulsion can be formed from a mixture of Tween 80 and Transcutol at HLB 14 (F19). It can be seen that the droplet size produced by HLB ranges from 10-200 nm.

2.3. Thermodynamic Stability Studies

The solubilization capacity of SNEDDS was large enough with the potential ability to create a thermodynamically stable system. Hence, a thermodynamic test is required. SNEDDS allocates stability by reducing the free energy of the interface and providing a mechanical barrier to coalescence and producing thermodynamically spontaneous dispersions, and the results are shown in Table 3.

Table 3. Result in thermodynamic stability studies of *E. palmifolia* extract-loaded SNEDDS.

HLB Value	Selected SNEDDS formulation	Heating-cooling Cycle		Freeze-Thaw Cycle	
		4°C ± 2°C	45°C ± 2°C	-20°C ± 2°C	25°C ± 2°C
14	F19	stable	stable	stable	stable
15	F35	stable	stable	unstable	unstable

The formulations involved in this stability test were F19 (HLB 14) and F35 (HLB 15). Nanoemulsion is thermodynamically stable and produced in the presence of oil, surfactants, and co-surfactants without phase separation, creaming or cracking. This differentiates nanoemulsion from macroemulsion, which is kinetically unstable and might result in phase separation [23].

3. DISCUSSION

In the past decade, much attention has been directed to lipid-based formulations, emphasizing improving and enhancing the solubility and oral bioavailability of poorly water-soluble. Ideally, SNEDDS transport a lipophilic drug in solubilized form and retain satisfactory solubilization through the gastrointestinal tract. Moreover, SNEDDS protect drugs against enzymatic degradation, foster supersaturation, surfactant-provoked membrane fluidity, and permeability enhancement that is often sufficient for drug absorption [19].

The oral route is the most comfortable and most convenient way of non-invasive administration. However, oral drug delivery may drug molecules that exhibit a poor aqueous solubility. Approximately 40% of the new chemical entities exhibit poor aqueous solubility and present a significant challenge to the modern drug delivery system, which leads to poor oral bioavailability. Apart from the oil, a surfactant is an essential component of SNEDDS [19]. The surfactant properties, such as HLB value, viscosity, and affinity for oil, strongly affect nanoemulsification and the droplets size [20]. The mixture of hydrophilic and lipophilic surfactants are used to form nanoemulsion with desired characteristics. HLB <10 is hydrophobic and could produce W/O emulsion, while HLB > 10 is hydrophilic and could produce O/W emulsion. The surfactant concentration plays a role in the formation of droplets size in nanometric [21]. The bigger the ratio between hydrophilic and lipophilic surfactants, the higher its HLB. The proper mixture of surfactants with lower and higher HLB might produce stable nanoemulsion, even when diluted. Additionally, the proper mixture might lower the interfacial tension, thereby facilitating the dispersion process and forming a flexible film that could readily deform around the droplet [22].

In the self-nanoemulsifying systems, the free energy required to form an emulsion was deficient (it was not practically measured), thereby allowing spontaneous formation of an interface between the oil droplets and water. Moreover, because the drug released will be in nano size, it will increase the effective surface area for dissolution and ultimately. The Miglyol 812 oil used is a medium-chain triglyceride (MCT), with higher solvent capacity than long-chain triglycerides, and not quickly turn rancid due to oxidation [13]. Besides, MCT can increase intestinal absorption [23]. This advantage of MCT over LCT is due to each fatty acid's configuration, especially those with a binding bond. The double bond strength is more with the cis isomer causing the chain to bend, thereby limiting the configuration of fatty acids [24].

Optimum clarity is achieved when the transmittance value is very high or closer to 100% [27]. Formulation with transmittance values higher than 80% is recognized as emulsions with nanosized for the droplets of oil in water. Also, the size of the dispersed phase dramatically influences the appearance of nanoemulsions. When the nanoemulsion system has a minimal globule size through which light could pass, the solution's color is transparent, and the resulting transmittance is even higher. Usually, this is compared with distilled water because it does not have particles that hold light transmission. Therefore, the light passes through without a scattering effect and a transmittance value of 100% [25].

In selecting the *E. palmifolia* SNEDDS formulation, the best is the high transmittance percentage, usually > 80% and the highest HLB. Furthermore, the use of surfactants in SNEDDS formulations needs to be as minimal as possible to minimize the risk of unwanted effects due to its excessiveness. The emulsification time is the ease with which SNEDDS form an emulsion in the body. Little emulsification time is caused by surfactants and co-surfactants, which could immediately form the interface layer of oil and water. Also, co-surfactants play a role in emulsification time rather than reducing the size of the droplet. It could slip and form a space between surfactants, thereby forming a swollen structure but with high fluidity and the ability to form nanoemulsion faster [27]. The pH range of the formulation was within 7.20–9.10. Viscosity is a measure of the resistance of a liquid to flow. Hence, the higher the viscosity of the formulation, the higher the resistance. The profile of pH and viscosity is shown in Table 2.

The droplet size analysis is a critical factor in self-emulsification because it determines the speed and ease with which the drug is optimally absorbed and the stability of the emulsion formed. The results of the test are shown in Table 2. The droplet size is a critical parameter of SNEDDS evaluation. The smaller its size, the larger the area of absorption, and the faster the drug release. Small droplets also provide a larger surface area, allowing pancreatic lipase to hydrolyze and promote more drug release [26]. In general, the SNEDDS formulations have droplet sizes ranging between 10 - 200 nm. This is consistent with past literature showing a particulate size value of 10 – 200 nm for a successful SNEDD process. The smaller the size SNEDDS will further increase vulnerabilities and its distribution in dissolution media.

Interest in herbal drugs from natural sources has grown in recent years, and herbal products are being used as alternative and complementary medicines worldwide. Herbal drugs have been utilized from time immemorial and are still part of modern medicine. Therefore, in developing this self-nanoemulsion drug delivery system, stability testing is required to ensure that *E. palmifolia* in the SNEDDS using oil, surfactant, and co-surfactant can remain stable in its use and storage within a certain time and temperature.

SNEDDS stability test was carried out to guarantee drug stability in a long time of storage. Thermodynamic stability occurs when free energy of colloidal dispersion (formation of droplets in water) is lower than free energy phase separation (oil and water) [20]. The form of the instability of the emulsion system is characterized by aggregation of the dispersion phase, the formation of layers, and the separated layer of the dispersion phase [27].

The thermodynamic stability is considered an important physical parameter to be fulfilled by the optimum SNEDDS formulation because it describes the durability of a product according to certain limits during storage and uses. The SNEDDS formulation was evaluated using a heating-cooling cycle test and freeze-thaw cycle to investigate the product's stability from the nanoemulsion preparations [27]. The tests showed that F19 and F35 remained stable after being stored at a temperature of 4°C ± 2°C and 45°C ± 2°C. The freeze-thaw cycle test was performed to observe the thermodynamic stability due to the SNEDDS heating cooling cycle test through visual observation. The tests showed that F19 remained stable after being stored at the temperature of -20°C ± 2°C and 25°C ± 2°C. However, F35 was unstable in the freeze-thaw cycle.

The formulations that passed the characterization were subjected to a thermodynamic stability test. It was observed that increasing the oil ratio enhances micelle formation, which consequently increases the stability. Only 1 of the 2 formulations passed the thermodynamic stability test and were able to freeze-thaw, and heating-cooling cycles as well as no drug precipitation. The formula with optimal stability is F19 with a ratio of 2: 7: 1 consisting of Miglyol 812, Tween 80, Transcutol, and PEG 400. Tween 80 as a non-ionic surfactant is not easily affected by acid and electrolyte conditions so that it remains active as a surface layer between oil and water. In a nanoemulsion system non-ionic surfactants stabilize the self nanoemulsion system. The surface charge comes from the adsorption of ions in the water phase or due to friction between the droplets and the dispersing medium. The ions that are adsorbed on the droplet surface form an electric double layer, resulting in a repulsive force between particles that hinders aggregation. In the O/W nanoemulsion system containing non-ionic surfactants, the surfactants form a film layer on the droplet surface. The film layer will prevent the droplets from combining in the dispersing medium [22].

4. CONCLUSION

The present study has demonstrated of SNEDDS based on the HLB of mixed surfactant. The change in HLB affected the characteristics and stability. The use of Tween 80/Transcutol resulted in SNEDDS that can produce nanosized emulsions after dispersion and good stability. The selected formulation was F19 (HLB = 14) with a ratio of 2:7:1 consisting of Miglyol 812 (2), Tween 80 (63.52), transcutol (6.48), and PEG 400 (1). Furthermore, the results showed that the HLB approach in SNEDDS formulations could produce optimal formulations. Its characteristics showed transmittance percentage of 97.97 ± 0.17%; emulsification time on AIF media 52.00 ± 0.03 seconds; AGF 50.00 ± 0.03 seconds; pH 8.90 ± 0.06; Viscosity 32.81 ± 0.41 cps; droplet size 12.08 nm.

5. MATERIAL AND METHODS

5.1. Extract Preparation

The *E. palmifolia* was extracted three times with 500 mL ethanol at ambient temperature through sonication Q2400 (Sonica, USA) at 10 min interval. About 25 g 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).

5.2. 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.

5.3. Materials

Miglyol 812 was purchased from Sigma Aldrich. Tween 80 and polyethylene glycol (PEG) 400 were purchased from Merck. Tween 20 and Span 20 were purchased from Bratachem. Transcutol was gift from Gattefose.

5.4. Surfactants Selection and Preparation SNEDDS

Two hydrophilic surfactants (i.e., Tween 20 and Tween 80) were mixed with two lipophilic surfactants (i.e., Span 20 and Transcutol) to formulated four binary surfactant combinations with hydrophilic lipophilic balance (HLB) ranging from 11 to 15 as shown in Table 4. HLBmix of each surfactant was calculated by using the following equation: HLBmix= fAHLBA + fBHLBB. HLBA and HLBB are HLB value of surfactant A and surfactant B; and fA and fB are the fraction weight of surfactant A and surfactant B, respectively.

In SNEDDS formulation, the HLB method is used as a starting point to acquire excellent emulsification characteristics. More than one surfactant is usually blended to get desirable HLB. However, the selected surfactants should have good miscibility with other SNEDDS formulation components to produce a stable and homogenous system. Also, the surfactant should have relatively low toxicity for oral administration.

SNEDDS formulation selection is made using HLB, made up of 20 g/formulation with the ratio of oil: surfactant:co-surfactant (1:7:2 and 2:7:1). The SNEDDS formulation does not use the aqueous phase's addition because SNEDDS is an isotropic mixture consisting of oil, surfactants, and co-surfactants. The formation of SNEDDS is characterized by the formation of nanoemulsions after SNEDDS contact with gastric fluid in less

than 2 minutes. Usually, a successful mixture is characterized by the formation of clear and transparent solutions with no phase separation. Then, the formulations showing good results is used to begin the manufacturing and characterization of *E. palmifolia* extract-loaded SNEDDS. The oil phase used is Miglyol 812, and the design formulation with the amount of the surfactant mixture is presented in Table 4.

5.5. Preparation *E. palmifolia*-loaded SNEDDS

The SNEDDS preparation was made using 50 mg *E. palmifolia* extract added to 20 g of SNEDDS. This was continually stirred for 1 min using a magnetic stirrer (Heidolph, Germany) at a 300 rpm speed at 25°C ± 2°C, after which it was characterized.

Table 4. Formulation ratio of *E. palmifolia* extract-loaded SNEDDS.

Rasio HLB mix	Miglyol 812	Tween 20/Transcutol	Tween 80/Span 20	Tween 20/Span 20	Tween 80/Transcutol	PEG 400
Ratio Formulation 2 : 7 : 1						
11	2	38.08/31.92 (F1)	26.25/54.75 (F6)	20.74/49.26 (F11)	44.07/25.93 (F16)	1
12	2	43.68/26.32 (F2)	37.20/32.80 (F7)	29.40/40.60 (F12)	50.56/19.44 (F17)	1
13	2	49.28/20.27 (F3)	48.13/21.87 (F8)	38.02/31.98 (F13)	57.04/12.96 (F18)	1
14	2	54.88/15.12 (F4)	59.1/10.90 (F9)	46.67/23.33 (F14)	63.52/6.48 (F19)	1
15	2	60.48/9.52 (F5)	70.00/0.00 (F10)	55.31/14.69 (F15)	70.00/0.00 (F20)	1
Ratio Formulation 1 : 7 : 2						
11	1	38.08/31.92 (F21)	26.25/54.75 (F26)	20.74/49.26 (F31)	44.07/25.93 (F36)	2
12	1	43.68/26.32 (F22)	37.20/32.80 (F27)	29.40/40.60 (F32)	50.56/19.44 (F37)	2
13	1	49.28/20.27 (F23)	48.13/21.87 (F28)	38.02/31.98 (F33)	57.04/12.96 (F38)	2
14	1	54.88/15.12 (F24)	59.10/10.90 (F29)	46.67/23.33 (F34)	63.52/6.48 (F39)	2
15	1	60.48/9.52 (F25)	70.00/0.00 (F30)	55.31/14.69 (F35)	70.00/0.00 (F40)	2

5.6. Characterization of *E. palmifolia*-loaded SNEDDS

5.6.1. Transmittance Test

The emulsion formed in the previous stage was observed using 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 [14].

5.6.2. 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 4). Usually, the self-emulsification is the time required by the pre-concentrate to form 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 [15].

Table 4. Formulation of artificial gastric fluid (AGF) and artificial intestinal fluid (AIF).

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

5.6.3. pH

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 [16].

5.6.4. 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 placed in the sample cup. This is to ensure it is bubble-free and evenly spread. The sample cup was also reassembled on viscometer, turned on, and then left until the reading was stable. Three repeated readings were performed for the sample [16].

5.6.5. Droplet Size

The droplet 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 distilled 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 indices between the oil and water phases) was used. Three repeated readings were performed for the sample.

5.7. Thermodynamic Stability Studies

5.7.1. 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 hours. 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 resulting nanoemulsion was observed for instability problems [17,18].

5.7.2. 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 hours. The formulation was diluted with double distilled water (1:25), and resulting nanoemulsion was observed for instability problems [19].

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