



Formulation and Characterization on Self Nanoemulsifying Drug Delivery System of *Eleutherine Palmifolia* Extract Employing Short, Medium, and Long Chain Triglyceride

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Abstract

Eleutherine palmifolia (*E. palmifolia*) is a common plant used as an anticancer drug in Kalimantan, Indonesia. The active compound present in it is naphthoquinone, which has low water solubility and needs to be formulated into SNEDDS preparations to improve its dissolution and bioavailability in the body. The dispersion method is used in the preparation of Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) and PEG 400 co-surfactants. Furthermore, this study aims to ascertain the effects of oil on the SNEDDS preparations. The results showed that the effect of the preparations on olive oil, VCO, and oleic acid in the organoleptic test produced dark red, clear, and non functional emulsion. The average particle sizes obtained in the AGF media were 61.10, 774.33, and 211.70 nm; in AIF media it amounted to 211.70, 123.65, and 830.22 nm. The pH value for each formula was 6.8, while the average emulsification time for the AGF media was 24.10, 22.53, and 25.75 seconds, and, lastly, for the AIF media, they were 20.96, 19.62, and 23.74 second.

Keywords: *Eleutherine palmifolia* (L.) Merr), self nanoemulsifying, SNEDDS, olive oil, VCO, oleic acid

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■ Introduction

Drugs pathways to action are highly complicated due to the complex cellular networks in an organism, which serve as guides for drug molecules to locate their targeted sites. Therefore,

accurate drug delivery reduces the undesirable side effects on non-target organs [1]. Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) is a medicine made from the isotropic mixtures of oil, surfactants, and cosurfactants that spontaneously form oil nanoemulsions in the

digestive tract and produce nanometer droplet sizes. SNEDDS have several advantages; namely, the emulsification of water-insoluble medicine into nanometer-sized droplets improves the bioavailability of oral drugs, and it is easily stored due to its thermodynamic stability. The presence of oil on the drug accelerates it through the gastrointestinal channel, thus minimizes irritation [2, 3].

The oil component in the preparations has a role in determining the quantity of emulsion formed and acts as the primary carrier of active substances [4]. The olive oil composed of most unsaturated fatty acids (oleic, linoleic, and linolenic), micronutrients, especially vitamins (A, E, and β -carotene), and microconstituents (compounds such as phenolic or chemicals present in unsaponified fraction) and squalene obtained from *Olea europaea* [5]. VCO is a triglyceride which consists of medium-chain dominant fatty acids 5.21%, caprylate 48.66%, and myristate 17.82 % [6]. Moreover, comprise of moderate-chain fatty acids that contain caprylic (C8), capric (C10), and lauric acid (C12), and lastly, the active substances namely medium-chain triglycerides (MCT), which prevent disease occurrence, and useful in the treatment of dangerous illnesses such as cancer and HIV/AIDS [6]. Oleic acid (cis-9-octadecenoic) is an unsaturated and long-chain (C18) fatty acid with a double bond between the 9th and 10th carbon atoms widely used as oil in SNEDDS formulation.

The active ingredient used is the extracted *E. palmifolia* with many benefits, one of which is colon anticancer. It also contains naphthoquinone with its derivatives such as elecanacine, eleutherine, eleutherol, and eleuthernone [7]. Napthoquinones as an anticancer and antioxidant have low water solubility, which needs to be formulated into the SNEDDS preparations to increase its dissociation and bioavailability in the body.

This study aims to determine the effect of olive oil, VCO, and oleic acid phase variations on SNEDDS preparations of *E. palmifolia*.

■ Experimental

Extraction *E. palmifolia*

E. palmifolia was extracted three times with 500 mL of ethanol by sonication (per 10 min, at ambient temperature). An extracted sample of 25 grams was dissolved in ethanol 96% 500 mL (ratio 1:20). The collected filtrate was then separated from its solvent using a rotary evaporator.

Formulation SNEDDS Extract of *E. palmifolia*

The method for making SNEDDS extract of *E. palmifolia* is a solid disperse, with formula as shown at Table 1.

Table 1. Formulation SNEDDS Extract of *E. palmifolia*

Ingredients	Formula 1 (F1) (g)	Formula 2 (F2) (g)	Formula 3 (F3) (g)
<i>E. palmifolia</i>	0.05	0.05	0.05
Tween 80	6.75	6.75	6.75
PEG 400	2.25	2.25	2.25
Olive oil	1.00	-	-
VCO	-	1.00	-
Oleic acid	-	-	1.00

The SNEDDS preparations included the mixture of the following ingredients: oil, surfactant, and cosurfactant. Then they were stirred using a magnetic stirrer 400 rpm for 10 min. After this, *E. palmifolia* was dissolved with ethanol and added into the formula.

Characteristics of *E. Palmifolia* Extract SNEDDS

Organoleptic

The sense of sight was used to deduce the color while instinct of smell was used for determining the odor of the preparations

Particle size

To ascertain the size of SNEDDS preparations, nanoemulsion measurements were carried out using a Particle Size Analyzer (PSA) (Nanowave II Microtec[®]). Each 3 mL of

SNEDDS preparations were placed in a cuvette with distilled aquadest, AIF, and AGF.

Transmittance

The total of 100 μ L of the formula was added to distilled water up to a volume of 50 mL [9]. Homogenization of the mixture was carried out using a vortex for 30 second. Transmitted SNEDDS was measured using spectrophotometry at a wavelength of 650 nm with a blank aquadest to determine its clarity [9].

Nanoemulsion type

Nanoemulsion type testing used a dilution method. This was performed by dissolving the sample into the water (1:100) and oil (1:100). When it completely dissolved in distilled water, the nanoemulsion was classified as the oil in water (O/W) type. In contrast to its dissolution in the oil phase, which was regarded as water in oil (W/O) type [10].

pH

The pH was measured using its meter, which was adjusted to the value of the small intestine since it is the site for the absorption of preparations [9].

Emulsification time

The emulsification time calculation was carried out on the nanoemulsion of herbal extracts in three different media aquadest, artificial gastric fluid without pepsin, and intestinal fluid lacking pancreatin. A medium of 500 mL was conditioned at 37 °C with a magnetic stirrer at a speed of 100 rpm. 1 mL SNEDDS formula was quickly dropped into the media. The following observations were made from the beginning of the drop, namely visual, transparency, and phase separation. The formation of nanoemulsion was confirmed by the complete dissolution of the preparations [10].

■ Results and Discussion

Organoleptic

The organoleptic test was based on descriptive observations. The results of SNEDDS

extract without *E. palmifolia* formula 1, 2, and 3 produced a bright yellow color, clear, odorless, no phase separation, and homogeneous. While those with the formula produced a bright red color, clear, odorless, phase separation, and no homogeneity. Meanwhile, the desired specifications were expected to be no change in phase, bright colour, odorless, and homogeneity [11].

Particle size

The particle size of the SNEDDS extract of *E. palmifolia* formula 1, 2, and 3 added to aquadest, AGF and AIF liquids are still undetermined. At the same time, those mixed with distilled water have an average size of 1000 nm, which was the highest value from the various the media since the preparations were emulsified into nanometer size in the gastric and intestinal fluids (AGF/AIF). However, a separate test was carried out that produced a smaller particle than the preparations in distilled water. It was observed that formula 2 had an average particle size smaller than 1 and 3. The nanoemulsion of medium-chain triglyceride (MCT) had mean droplets compared to olive oil and oleic acid long-chain triglycerides (LCT). While the emulsions of MCT had an insignificant smaller particle than those of long-chain triglyceride LCT. The short-chain alkane can reduce the hydrophilic lipophilic balance (HLB) of both triglycerides [13].

Based on statistical analysis, in the normality test using Shapiro Wilk, with three media (aquadest, AGF, and AIF), a significant value > 0.05 was obtained, which showed that particles were evenly distributed. The homogeneity test also had the same value, which indicated that the particles were homogeneous. Then one way ANOVA test with a value of < 0.05 was obtained, which signifies separation between the particle and the media, and the post hoc LSD test, with a value < 0.05 , which concluded that there was a difference between formula 1 and the others. Results of SNEDDS particle test presented in Table 2 and Figure 1.

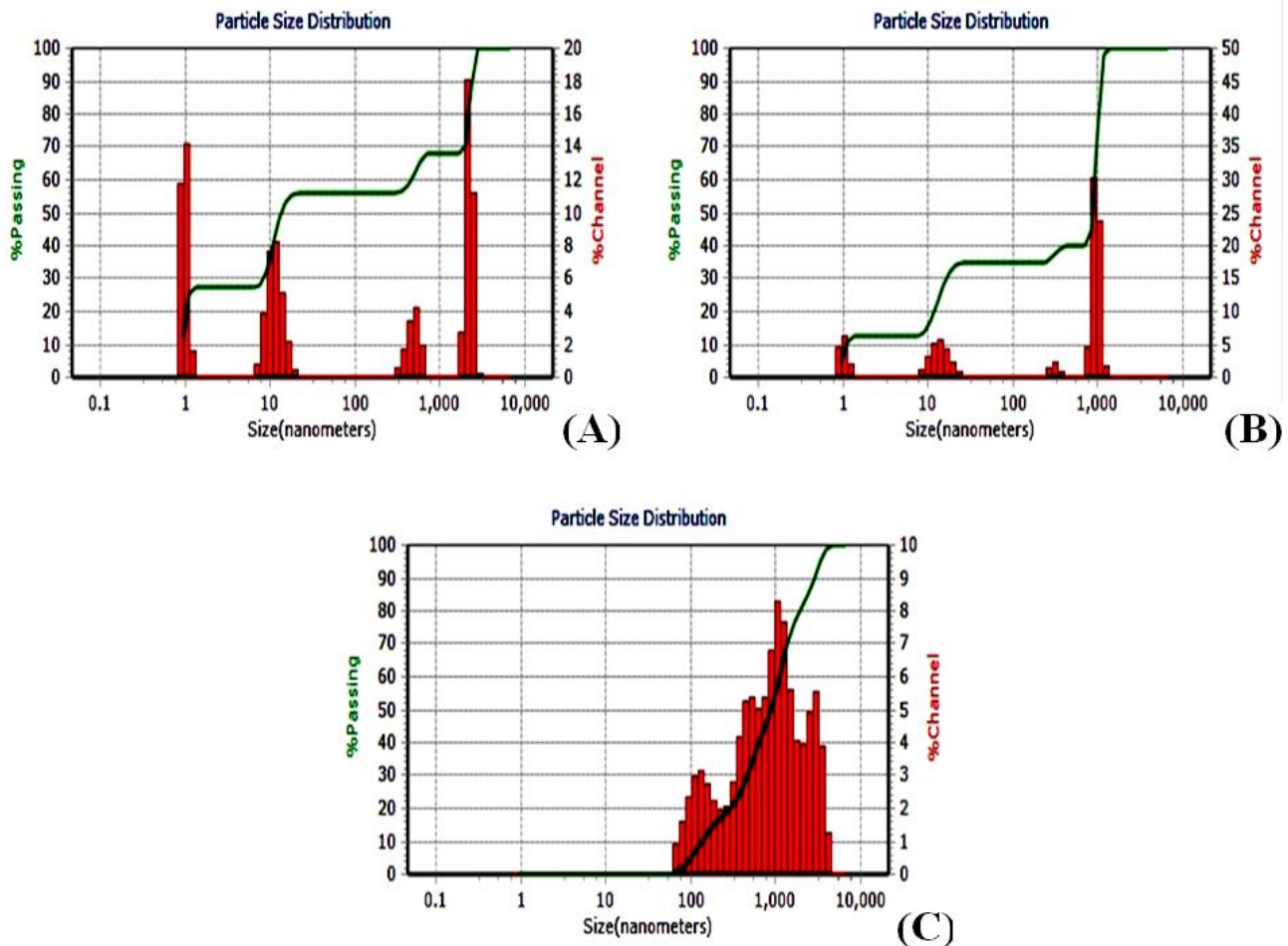


Figure 1. Diagram of particle size using Nanowave II (Microtec, USA) (A) olive oil (B) virgin coconut oil (C) oleic acid

Table 2 Result of particle size on various media

Media	Formula	Particle size \pm SD
Aquadest	Formula 1	696.67 \pm 77.75
	Formula 2	555.00 \pm 76.45
	Formula 3	1147.33 \pm 47.09
Artificial Gastic Fluid (AGF)	Formula 1	352.10 \pm 86.47
	Formula 2	61.10 \pm 08.66
	Formula 3	774.33 \pm 138.41
Artificial Intestinal Fluid (AIF)	Formula 1	211.70 \pm 30.26
	Formula 2	123.65 \pm 2.33
	Formula 3	830.33 \pm 64.61

Transmittance

Based on table 3, formulas 1, 2, and 3 had average values of 87.65% \pm 0.10, 92.91% \pm 0.18, and 56.19% \pm 0.28. The value of 80% above indicated a transparent emulsion; therefore, the

bigger the particle size formed by oil in water, the more visible the phase [14]. These results showed the need for different transmittance, due to the diverse oil phases which affected the droplet size and the stability of the nanoemulsion formed, and also acted as a carrier that dissolved the active substance (lipophilic) and formed oil droplets with the help of surfactants and cosurfactants [15].

The size of the oil dispersed influenced the appearance of the emulsion. As a result, the larger the size of the droplet, the higher the transmittance value, and was estimated to reach the value of a nanometer. The emulsion system appeared to be transparent when passed through light, and the resulting transmittance value was even higher.

Tabel 3. Result of Transmittance test SNEDDS *E.palmifolia* Extract

Formula	Transmittance (%) \pm SD
Formula 1	87.65 \pm 0.10
Formula 2	92.91 \pm 0.18
Formula 3	56.19 \pm 0.28

Nanoemulsion type

The results showed that the formulas 1, 2, and 3 were soluble in distilled water but not in oil. The nanoemulsion and the HLB of the surfactant used were classified as the oil in water (O/W) type. The surfactant and HLB used had an average transmittance value of 80 and 15, respectively. Therefore, the HLB 15 indicated increased solubility of the formulas in water, since most of their components were hydrophilic or polar. However, there was the presence of a few hydrophobic[12].

pH

Based on the results, the pH 6.8 value obtained corresponded with that of the intestine and eventually led to the high absorption of the preparation, which was a formulation between oils, surfactants, and cosurfactants that were contained in drugs. Firstly, it entered the digestive tract and mixed with the intestinal fluid. After this, spontaneous emulsification occurred and produced nanometer sizes, which were finally absorbed.

Emulsification time

The time of emulsification determines how quickly the emulsion is formed. This formation is accelerated by direct contact with gastric fluid, an essential parameter in the preparations. It is also formed spontaneously without any shaking process.

Table 4 Result of Emulsification time test SNEDDS *E. palmifolia*

Media	Formula	Emulsification time \pm SD (second)
Aquadest	Formula 1	23.12 \pm 0.33
	Formula 2	22.19 \pm 0.54
	Formula 3	43.97 \pm 0.34
Artificial Gastric Fluid (AGF)	Formula 1	24.10 \pm 0.21
	Formula 2	22.53 \pm 0.22
	Formula 3	25.74 \pm 0.20
Artificial Intestinal Fluid (AIF)	Formula 1	20.96 \pm 0.42
	Formula 2	19.62 \pm 0.35
	Formula 3	23.74 \pm 0.27

Based on Table 4, the preparations had an excellent emulsification time that was less than 2 min. It showed that the size of the particles formed was already in nano size, since the clearer the emulsion, the smaller its content size [2]. The results obtained were following the stipulated time of less than 2 min which led to the formation of nanoemulsion within a short period and accelerated by the presence of surfactants and cosurfactants, which have a mechanism of reducing the interface tension of water and oil, by forming a swollen structure with high fluidity that hasten the formation of nanoemulsions [18].

Conclusion

The effects of olive oil (formula 1), VCO (formula 2), and oleic acid (formula 3) on SNEDDS preparations of *E. palmifolia* produce a dark red, clear, and odorless emulsion. There is no separation and homogeneity for oil in water (O/W) type nanoemulsion. The average particle sizes obtained in AGF media were 61.10, 774.33, and 211.70 nm, in AIF media were 211.70, 123.65, and 830.22. While the percentage of transmittance obtained was 87.65, 92.91, and 56.51%. The pH value for each formula was 6.8 while the average emulsification time for the aquadest media was 23.12, 22.19, and 43.97 second, for the AGF media were 24.10, 22.53, and 25.75 second, and lastly for the AIF media were 20.96, 19.62, and 23.74 second.

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