Optimization Formula of SNEDDS Dosage from Ethanol Extract of Turmeric (*Curcuma domestica*) With Waste Oil of Eel (*Anguilla spp.*) as A Carrier

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Abstract. Turmeric have a lot of advantages for us, usually for treatment a lot of disease, and it has efficiency as a drug. But turmeric extract have very low solubility and it cause minimal oral bioavailability. While nutrition of waste oil of Eel (*Anguilla spp.*) are higher than the other fish, because this waste oil have palmitic acid, linoleic acid, and oleic acid that usefull for treatment some disease. So, in this research, turmeric extract made by Self-Nanoemulsifying Drug Delivery System (SNEDDS) method with waste oil of Eel (*Anguilla spp.*) as a carrier to overcome solubility of turmeric extract. In this research, the turmeric was extracted with maceration method using ethanol as a solvent. This extracting showed high average rendemens are 6.62-8.19 %. Percentages of curcumin for this turmeric extract was calculated by TLC densitometry, shows 2.99 % b/b. This extract had the optimization of the formulation of the composition of surfactant by Tween 80, cosurfactant by PEG 400, and waste oil of Eel (*Anguilla spp.*) as a oil carrier. After done some tests, include loading dose test on the selected optimal formulation, then we had determine that quality. It included by counting emulsification time, observations of the particle size, its distribution, and zeta potential of nanoemulsions using PSA. The results of SNEDDS optimal dosage was calculated by Design Expert 7 had a mass (gram) ratio of waste oil of Eel (*Anguilla spp.*):Tween 80 (surfactant): PEG 400 (cosurfactant) = 0.55 :4.10:0.35 added with loading dose 0.5 g of Turmeric extract in 5 g system of SNEDDS. The result had a transmittance value of 91.48-92.17 % with emulsification time less than 30 seconds and it classified type A (Sakhi et al, 2013). Particle size of the chosen SNEDDS nanoemulsion turmeric extract that calculated by PSA, shows 11.9 nm and with polydispersity index are 0.118 droplet and zeta potential of -11.8 mV.

1. Introduction
The increasing of human population is the one problem in our life because it is not followed by increasing of human health [1]. So, we have to increase healthy service, availability drugs completely, efficiently drugs, safety drugs with affordable price. The main problem of herbal medicine are poorly water soluble and the oral delivery [2] [3]. SNEDDS is one of the medical preparation method to increase the absorption of the lipophilic drugs [4], because SNEDDS are isotropic mixtures that consist of oil, surfactant, co-surfactant and drug in form oil in water emulsion. This forms is a good
mode to deliver poorly soluble drugs orally by increasing its bioavailability and stability [5] [3]. Turmeric is a rhizomes belonging to a ginger family (Zingiberaceae) that contain important bioactive component called curcumin [6] [7]. It has bioactive components with regard to potential as a herb medicine [6] [8] [1], but because of its low bioavailability, its usefulness, and attainable blood levels associated to its consumption is laced with doubt [9]. While fatty fish specially waste oil Eel (Anguilla spp.) are considered to be of great nutrition [10]. This waste oil have palmitic acid and oleic acid that can reduce cholesterol in blood [11]. This is due to their high natural component consist of essential n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid and docosahexaenoic acid. These fatty acids have been shown to have potential benefits for human health [12][13]. Nanoemulsion form is a good choice that is expected to increase solubility and oral bioavailability [14][15][16] of turmeric extract ethanol fraction and waste oil of Eel (Anguilla spp). When they are combined into water phase in a slow agitation condition, spontaneously, it will form nanoemulsion of oil phase in water (O/W) [2]. The successfulness of this research is expected to be a nanoemulsion formula of ethanol fraction from turmeric extract with waste oil of Eel (Anguilla spp) as a carrier to be a good product as alternative one of herb medical with good bioavailability.

2. Experimental
2.1 Equipment and Materials
Turmeric rhizome and waste of Eel (Anguilla spp) were obtained from UNAGI Surakarta, Indonesia. Component of optimization of SNEDDS: aquabidest, aquadest, chloroform, ethanol, methanol, PEG 400, tween 80 (Brataco), Component of artificial gastric fluid (AGF) consist of NaCl, HCI 37 %, and aquades. Equipment which were used are blender, glass made tools (Pyrex), oven, stopwatch, waterbath (Grant), rotary evaporator (RVO 400 SD Bocco Germany), vortex (Maxi Mix II Thermolyne), magnetic stirrer (IKA C-MAG HS7), analytical balance (KERN ALJ), yellow tip (Kan Jian), sonicator (Branson 1510), flacon glasses, spectrophotometer UV Vis (Perkin Elmer Lambda 25), micropipet 50-1000 μL, PSA (Horiba SZ-100), and analytical scale (Metler Teledo).

2.2 Procedure
2.2.1. Maceration of Ethanol Fraction from Turmeric Extract and Waste Oil of Sidat (Anguilla spp). Turmeric powder was extracted with ethanol solvent (1:5) for 5-7 days. Then turmeric’s macerate was filtered to separate it from simplisia powder. Thus, the filtrat was evaporated by rotary evaporator for 1 hour until it become concentrated in 70 °C temperature and 3 rotary speeds. Then rendemens of turmeric extract was calculated. Percentages of curcumin was calculated by TLC-densitometry. Waste of Eel (Anguilla spp) was extracted by maceration method for 3-4 days with added 400 mL chloroform and 400 mL methanol, mixed until it became homogeneous mixture. Macerate was filtered to separate it from sludge. Thus, evaporate it by rotary evaporator in 60 °C temperature for 1 hour and 3 rotary speeds to be concentrated oil.

2.2.2. Optimization of surfactant, cosurfactant, and oil composition of SNEDDS-Turmeric Extract. The higest transmittan value was choosen from formulas of oil: surfactant: cosurfactant are 1:1:1 until 1:8:1. The selected formula was optimized and selected by using Design Expert 7 based on transmittan value and emulsification time in AGF, aquadest, and aquabidest.

Candidate of SNEDDS formula was taken 100 μL and added with aquades becomes 50 mL. Thus, it was homogenized by using vortex for average 30 minutes. The successfulness of making of nanoemulsion showed homogeneous emulsion and give clear visual. The transmittance of emulsifying formula of SNEDDS was measured by spectrophotometer on the length wave 650 nm with aquades blank to determine the clarity level.

Emulsification time was done by dropped quickly 1 mL SNEDDS formulation of turmeric extract in 500 mL medium AGF without pepsin, aquadest, and aquabidest, which were placed in 37 °C temperature on the magnetic stirrer with speed about 120 rpm. The observation was done by the time needed from the first drop until it forms emulsion using stopwatch. Nanoemulsion was formed which was dissolved in the medium perfectly [15].
2.2.3. SNEDDS formula selection.

Then, loading dose was done by added turmeric extract of ethanol fraction 250, 500, and 750 mg into 5 g optimal selected system component (carrier oil: waste oil of Eel (Anguilla spp.), surfactant: tween 80, and cosurfactant: PEG 400) which was appropriate with optimization results that had been done.

The mixture was sonicated for average 15 minutes to be homogeneous system, then it was placed in 45 °C temperature for 10 minutes. The loading dose was chosen by based on the higher dose of turmeric extract on the homogenous formulas. The result of the mixture was keep for 24 hours in the room temperature, freeze temperature, and 45 °C temperature to determine that homogenity. The homogeneous formula is the selected formula for SNEDDS [4].

Particle size, size distribution, and zeta potential was measured using Particle Size Analyzer (PSA).

3. Results and Discussion

3.1. Maceration and curcumin content of turmeric extract

Turmeric extract was macerated by ethanol as a solvent (1:5). This extract had high rendemens, it showed between 6.62-8.19%. It contained 2.99% w/w of curcumin level. Curcumin is a non polar liposoluble compound. It dissolved into semi polar solvent, one is ethanol as a good extractant. The high concentration of ethanol showed that a lot of curcumin can be dissolved in this solvent [7].

| No | Mass of Turmeric Powder (g) | Volume of Ethanol (mL) | Mass of Turmeric Extract (g) | Percentage of Rendemen % |
|----|----------------------------|------------------------|------------------------------|--------------------------|
| 1  | 151.00                     | 755.00                 | 10.00                        | 6.62                     |
| 2  | 67.79                      | 338.95                 | 4.75                         | 7.01                     |
| 3  | 150.57                     | 752.85                 | 11.05                        | 7.34                     |
| 4  | 111.8                      | 559.00                 | 8.5                          | 7.60                     |
| 5  | 118.5                      | 592.5                  | 9.7                          | 8.19                     |

3.2. Optimization of surfactant and cosurfactant composition of SNEDDS-Turmeric Extract

This aims to determine the right composition ratio of surfactant and co-surfactant which is able to produce a homogeneous formula after added with waste oil of Eel (Anguilla spp.) as a carrier. The results of surfactant and co-surfactant with waste oil of Eel (Anguilla spp.) (Table 2) showed that combination of waste oil of Eel (Anguilla spp.): tween 80 : PEG 400 with ratio 1:6:1, 1:7:1, and 1:8:1 were able to produce a homogeneous mixture.

| Ratio composition | Composition (g) | Condition       |
|-------------------|-----------------|-----------------|
|                   | Oil            | Tween 80 | PEG 400 |               |
| 1 : 1 : 1          | 1.67           | 1.67     | 1.67     | not homogeneous |
| 1 : 2 : 1          | 1.25           | 2.50     | 1.25     | not homogeneous |
| 1 : 3 : 1          | 1.00           | 3.00     | 1.00     | not homogeneous |
| 1 : 4 : 1          | 0.83           | 3.33     | 0.83     | not homogeneous |
| 1 : 5 : 1          | 0.71           | 3.50     | 0.71     | not homogeneous |
| 1 : 6 : 1          | 0.63           | 3.73     | 0.63     | Homogeneous(F1) |
| 1 : 7 : 1          | 0.55           | 3.88     | 0.55     | Homogeneous(F2) |
| 1 : 8 : 1          | 0.50           | 4.00     | 0.50     | Homogeneous(F3) |
The transmittance value of chosen formula (F1, F2, and F3) were measured using spectrophotometer to determine the highest value as a candidate for SNEDDS composition, showed that formula with the highest transmittance is formula F2 with average transmittance value is 96.966% (Table 3).

Table 3. Transmittance Homogeneous Composition

| Rasio composition of waste oil of Eel (Anguilla spp):Tween 80:PEG 400 | Condition         | Transmittance (%) |
|----------------------------------------------------------------------|--------------------|-------------------|
| 1 : 6 : 1                                                           | Homogeneous(F1)    | 65.697            |
| 1 : 7 : 1                                                           | Homogeneous(F2)    | 96.966            |
| 1 : 8 : 1                                                           | Homogeneous(F3)    | 96.647            |

The choosen formula was calculated by Design Expert 7 Programe to be eight runs with 0.55 g flat waste oil of Eel (Anguilla spp). Every formula from eight run of choosen formula showed high transmittance (more than 90%), while emulsification time in AGF, aquadest, and aquabidest are between 4-14 seconds (Table 4). It showed that the formulas was in nanoemulsion form type A [3], trusted as a good deliver for turmeric extract in formula SNEDDS.

Table 4. Transmittance Value and Emulsification Time from Eight Runs SNEDDS Formula

| Run | Transmittance (%T) | Emulsification Time in AGF (s) | Emulsification Time in aquadest (s) | Emulsification Time in aquabidest (s) |
|-----|--------------------|--------------------------------|-------------------------------------|--------------------------------------|
| 1   | 91.85              | 7                              | 10                                  | 12                                   |
| 2   | 91.97              | 5                              | 10                                  | 9                                    |
| 3   | 91.83              | 6                              | 10                                  | 8                                    |
| 4   | 92.23              | 6                              | 9                                   | 8                                    |
| 5   | 91.48              | 9                              | 8                                   | 12                                   |
| 6   | 91.75              | 4                              | 11                                  | 6                                    |
| 7   | 92.17              | 6                              | 9                                   | 14                                   |
| 8   | 91.91              | 12                             | 8                                   | 9                                    |

The eight runs formulas were choosen would be calculated by Design Expert 7 Programe to decide the optimum SNEDDS with turmeric extract formula. Linier models graph showed that higher level of surfactant and lower level of cosurfactan increased transmittan value (Figure 1) and emulsification time in aquadest (Figure 3) and aquabidest (Figure 4), but decreased emulsification time in AGF (Figure 2).
Design-Expert® Software

transmittan

Design Points

X1 = B: surfactant
X2 = C: cosurfactant

Actual Component

A: oil = 0.550

| surfactant (g) | cosurfactant (g) |
|---------------|------------------|
| 3.89          | 0.56             |
| 3.99          | 0.45             |
| 4.10          | 0.35             |
| 4.20          | 0.25             |
| 4.31          | 0.14             |

Figure 1. Linier Models of Two Component Mix of Transmittan Value

Two Component Mix

Transmittan (%)

Actual surfactant (g) 3.89 3.99 4.10 4.20 4.31
Actual cosurfactant (g) 0.56 0.45 0.35 0.25 0.14

Two Component Mix

Emulsification Time (s)

Actual surfactant (g) 3.89 3.99 4.10 4.20 4.31
Actual cosurfactant (g) 0.56 0.45 0.35 0.25 0.14

Figure 2. Linier Models of Two Component Mix of Emulsification Time in AGF
The flat composition of waste oil of Eel which was mixed with ratio formulation of surfactant and cosurfactant lead to the formation of SNEDDS, so there is a relationship between the droplet size and the concentration of the surfactant and cosurfactant are being used. By increasing the cosurfactant concentration could lead to droplets with smaller mean droplet size [2], but the other hand the mean droplet size may bigger with increasing surfactant concentrations, so the linier models above showed the optimum composition surfactant and cosurfactant to be homogeneous emulsion of SNEDDS formulas. The stabilization of the oil droplets depend on of the localization of the surfactant molecules at the oil-water interface. The interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration [2].

The highest desirability value of SNEDDS turmeric (Curcuma domestica) extract was 0.887 (Figure 5). This formula will produce optimum characteristic SNEDDS of turmeric extract. The
composition of optimum formulation of SNEDDS were 4.10 g of surfactant Tween 80; 0.35 g of cosurfactant PEG 400, and added 0.55 g flat of waste oil of Eel (Anguilla spp) (Table 3).

Based on solution that calculating by Design Expert 7 Programe, this optimization of SNEDDS increased the high transmittan value and decreased emulsification time in AGF, aquadest, and aquabidest (Table 5). Of course, the optimum composition with the highest desirability provided the best result for formulation SNEDDS with 500 mg loading doses of turmeric extract.

**Table 5. Solution of optimization SNEDDS turmeric extract (Curcuma domestica)**

| Waste oil of Eel (g) | Surfac. (g) | Cosurfac. (g) | Transmid value (%) | Emulsif. time in AGF (s) | Emulsif. time in aquadest (s) | Emulsif. time in aquabidest (s) | Desirability |
|----------------------|-------------|---------------|--------------------|--------------------------|-------------------------------|---------------------------------|-------------|
| 0.55                 | 4.10        | 0.35          | 91.90              | 6.88                     | 9.38                          | 9.75                            | 0.887       |

Characteristic of SNEDDS was measured using PSA, showed that the result of particle size is 11.9 nm (Figure 6), distribution size was 0.118 ( PI<1), and zeta potential was -11.8 mV (Figure 7).

It showed that formula has nanoeulsion particle size less than 100 nm and uniform particle size distribution (PI<1) [16]. Increasing composition of surfactant to cosurfactant ratio lead to decrease in mean droplet size [17]. Accordance with Savale’s research, showed that fatty acid medium chain until long chain supported stability of SNEDDS emulsion [15], in this riset using waste oil of Eel which had medium until long chain of fatty acid, had stability formula SNEDDS emulsion.

Turmeric extract which has low bioavailibility oral need an exact formula which is able to be absorbed in lumen gut, so it can make a lot of advantages for human healty. This research showed that the optimization SNEDDS with oil waste of Eel (Anguilla spp.) as a carrier was the exactly formulation as drug delivery system for turmeric extract (Curcuma domestica).
4. Conclusion
The results of this research showed that the optimum SNEDDS formula was waste oil of Eel (\textit{Anguilla spp}) tween 80 : PEG 400 (0.55 : 4.10 : 0.35) added 0.5 g turmeric extract in 5 g system SNEDDS. This formula had emulsification time value less than 30 seconds, the particle size value 11.9 nm, and zeta potential value -11.8 mV.

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