Int. j. adv. multidisc. res. stud. 2023; 3(4):670-677

Received: 13-06-2023
Accepted: 23-07-2023

International Journal of Advanced Multidisciplinary Research and Studies

ISSN: 2583-049X

The Effect of Surfactant Concentration to Particle Size of Herbal Imunity Nanoemulsion

[1] Dian Eka Ermawati, [2] Nisa Anikmah

[1, 2] Department of Pharmacy, Vocational School, Universitas Sebelas Maret, Surakarta, Indonesia

Corresponding Author: **Dian Eka Ermawati**

Abstract

Herbal imunity consists of ginger, turmeric, centella, and cinnamon, that act as immunostimulant agents. However, the infusion impractical and limited doses of the extract used, so it is necessary to develop drug delivery to resolve that problem. The Self Nano Emulsifying Drugs Delivery System (SNEDDS) technique is expected to increase the solubility, drug release, and absorption of active substances in the body, especially for low solubility of an active substance. SNEDDS consists of oil, surfactant, and co-surfactant. A surfactant is a substance that can reduce surface tension so that emulsion globules form in nanoparticle size. Tween 80 can produce a more transparent solution for oil-in-water emulsions than surfactants with low HLB values. This study aimed to determine the effect of surfactant concentration on the physical properties of nanoemulsion to obtain the smallest particle size. The formula consists of coconut oil-tween 80-propilen glycol of 1:7:1; 1:8:1; and 1:9:1 that incorporated extracts were 100, 200, and 300 mg. The physical tests included transmittance percentage, emulsification time on AGF media, phase separation, and stability test using the cycling test method. Nanoemulsion, then followed by Particle Size Analysis. The results showed that the greater surfactant concentration reduce transmittance value and particle size, but increase the emulsification time and phase separation. Formula with oil: surfactant: co-surfactant of 1:7:1 is a system that meets the requirement for herbal imunity with an optimal loading dose and small particle size compared to another formula. The extract's loading dose of 300 mg have a particle size of 17.06 nm and a polydispersity index of 0.32.

Keywords: Herbal Imunity, Immunostimulant, Surfactant, SNEDDS, Loading Dose

Introduction

Herbal medicine in Indonesian people is called Jamu. Jamu consists of medicinal plants that use to maintain health. Herbal medicine is considered safer than synthetic drugs, but its use still needs to be corrected in medicinal plants due to misinformation (Herman *et al.*, 2019) [16]. The composition of Imunity Jamu is Ginger, Turmeric, Centella, and Cinnamon, which are believed to maintain the body's stamina and immune system and prevent disease.

The immunostimulatory activity was found in ginger with a 100-200 mg/Kg BW (Hasanah *et al*., 2020; Hidayah & Indradi, 2020 [17]; Srikandi *et al*., 2020). Turmeric (Curcuma domestica Val.) contains curcumin, a class of phenolic compounds flavonoids (Tirtayani *et al*., 2022). Turmeric extract at a dose of 400-600mg/KgBB has activity as an anti-inflammatory (Meilina & Mukhtar, 2018). Cinnamon (Cinnamomum burmannii (Nees & T. Nees) Bl.) contains high amounts of total phenolics and total flavonoids that are potential as an antioxidant (Antasionasti & Jayanto, 2021). Based on research by Rafita *et al*. (2015), cinnamon extract with a dose of 320 mg/KgBB showed activity as an antioxidant. Herbs Centella asiatica (L.) Urb.) contains chemical compounds flavonoids that function as antioxidants (Khairunnisa *et al*., 2022 [22]; Rahayu *et al*., 2020). Centella asiatica extracts at a 400 mg/KgBW dose were reported to have antioxidant activity (Hernayanti & Lestari, 2020). The most active substances in the combination of ginger, turmeric, cinnamon, and centella herbs are flavonoids and curcumin. Flavonoid and curcumin compounds are reported to have antioxidant, anti-inflammatory, and immunomodulatory activities (Hanani, 2015; Hidayah & Indradi., 2020 [17]; Marcha, 2020; Puspitaningrum *et al*., 2017).

Herbal imunity drinks are impractical, limited doses of medicinal plants, have stability problems and are not suitable for diabetes patients. So, it is necessary to develop a delivery system for the herbal components that make up immunity jamu as an herbal medicine. SNEDDS (Self-Nano Emulsifying Drugs Delivery System) is an isotropic system composed of surfactants, co-surfactants, and oil. The SNEDDS technique aims to increase the solubility of active substances in the body and increase the speed of dissolution and absorption of active substances, especially for active substances with low solubility (Artanti *et al.*, 2021 [4]; Nugroho and Sari, 2018). The SNEDDS component, namely the oil, functions as a carrier for the active drug

substance, the surfactant commonly used is a non-ionic group because it is non-irritating. The use of single surfactants is not able to reduce the surface tension between oil and water, so a co-surfactant is needed that functions to help reduce the surface tension by increasing the mobility of the hydrocarbon tail, which causes greater oil penetration in the tail (Shoviantari *et al*., 2019) [37]. According to Date *et al*. (2010) [9], the components in SNEDDS, namely oil, surfactants, and co-surfactants, can influence the optimal formulation due to their physicochemical properties, concentration, ratio of each component, pH, emulsification temperature, and physicochemical properties of the drug. Surfactants affect the solubility of the active ingredient, the ability to load dose, and the particle size of the resulting nanoemulsion globules. Tween 80 can reduce the interfacial tension between the drug and the medium while simultaneously forming micelles that can carry drug molecules into the medium. Tween 80 is non-toxic and stable to pH (Zulfa *et al*., 2019) [44]. In addition, Tween 80 has a hydrophilic balance (HLB) value of 15, which is higher than Labrasol of 12. A higher HLB value will produce a clear solution than surfactants with low HLB values. Surfactants with high HLB values can facilitate the formation of oil-water-type nanoemulsions. The selection of the oil phase is based on the maximum solubility of the active substance in the oil phase. In contrast, the selection of surfactants and co-surfactants is based on the maximum solubility of the drug in them and the efficiency of emulsification of the oil phase (Akbar *et al.,* 2021).

Ethanol 70% was used as a solvent for extracting the active substances of flavonoids and curcuminoids in components of herbal immunity. Ethanol is an organic solvent that can attract non-polar compounds and polar compounds. Flavonoids are bioactive compounds generally soluble in polar compounds (Suhendra *et al*., 2019). In contrast, curcumin is a non-polar compound with low water solubility but is soluble in semipolar solvents such as ethanol and methanol. The higher the concentration of ethanol, low the polarity, so it is suitable for extracting non-polar curcumin (Popuri *et al*., 2013) [27]. Research results by Sugiandi *et al.* (2021) state that the curcumin content in water solvent is 0.14% lower than ethanol 50% solvent of 1.67%. Ethanol concentration above 70% resulted in decreased levels of total flavonoids. Based on research by Suhendra *et al.* (2019) reported the total levels of flavonoids in extracts with ethanol 70% of 90.91 mg QE/g extract. Meanwhile, on 80% and 90% ethanol concentrations, the total flavonoid content was only 71.15 mg QE/g extract and 49.59 mg QE/g extract. Research by Chew *et al*. (2011) stated that the level of total flavonoids with a concentration of ethanol solvent of more than 70% of Centella extract decreased. Based on these, in this study, ethanol 70% was chosen compared to ethanol 96% and water to extract the flavonoid and curcumin compounds as the main ingredient in herbal immunity).

Based on the formula optimization by Ermawati *et al.* (2020) [14]. The ratio of oil: surfactant: co-surfactant in the SNEDDS formula used is 1: 1: 1 to 1: 9: 1. Formula with a ratio of oil: surfactant: co-surfactant 1: 9: 1 meets the quality requirements for nanoemulsion preparations and has a particle size of 150.2 nm with a loading dose of 200 mg/5 gram. In this study, three formulas were used based on transmittance percent results. The three formulas have an oil: surfactant: co-surfactant ratio of 1: 7: 1, 1: 8: 1, and 1: 9: 1 to determine the effect of surfactant concentration on the ability to load dose and particle size of the immunity jamu components ethanolic extract.

Methodology

Materials

White ginger rhizome, Centella herb, turmeric rhizome, and Cinnamon (CV Herba Dream, Karanaganyar, Central Java, Indonesia), virgin coconut oil (CV Happy Green Garden, Jakarta, Indonesia), Tween 80 (Repackaged by Cipta Kimia, Surakarta, Indonesia), Propylene glycol (Repackaged by Essential Oils Lansida Group, Yogyakarta, Indonesia), Aquadest (Repackaged by UD Saba Kimia, Surakarta, Indonesia), HCl (Repackaged by CV Cipta Kimia, Surakarta, Indonesia), NaOH (Repackaged by UD Saba Kimia, Surakarta, Indonesia), NaCl (Repackaged by UD Saba Kimia, Surakarta, Indoensia), Ethanol 70% (Repackaged by Cipta Kimia, Surakarta, Indonesia), Dichloromethane (Repackaged by CV Easy Berkah, Bantul, Indonesia), Chloroform (Repackaged by Eduscientia, East Jakarta, Indonesia), Methanol (Repackaged by CV Cipta Kimia, Sukoharjo, Indonesia), Ethyl Acetate (Repackaged by Pharmapreneur, Depok), Ethanol 96% pro analytical Brand KGaA® Specification 1.00971.1000, n-hexane (Repackaged by Pharmapreneur, Depok, Indonesia), Curcumin Standard (Sigma Aldrich, USA), Quercetin Standard (Sigma Aldrich, USA), and Silica Gel 60 F254 TLC Plate Brand KGaA® Specification 1.05554.0001 (Merck, Germany).

Methods

Sample Preparation

Plant determination was carried out in the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret, Surakarta, Indonesia. Immunity Jamu component powders weighed 400 grams of Centella herb, 200 grams of Turmeric, 200 grams of Ginger, and 200 grams of Cinnamon, respectively. Then it was put into the maceration vessel, added 5 L of ethanol 70% solvent. The maceration process was carried out for five days, and the stirring process was treated daily to prevent saturated solvents. After maceration, evaporation is carried out at 40 - 50 °C to evaporate the solvent until a thick extract. The extract was tested for quality, including organoleptic and water content (Rahayu *et al.,* 2022) [31].

Phytochemical Detection by Spectrophotometer UV-VIS Method

Maximum wavelength

Total Flavonoids. The herbal extract was weighed accurately to 50 mg, added 0.3 mL of sodium nitrite 5%, waited for 5 minutes, added 0.6 mL of aluminum nitrate 10%, waited for 5 minutes, added 2 mL of sodium hydroxide 1.0 M, placed in a measuring flask of 10 mL, then read the absorbance at 510 nm (Nguyen *et al.,* 2012). Curcumin. The herbal extract was weighed carefully, 100 mg was put into a measuring flask of 10 mL, added 2 mL of ethanol 96% then vortexed, and sonicated for 60 minutes. The sample was then centrifuged, and the supernatant was taken. The supernatant was put into a 10 mL measuring flask, added 1.5 mL of ethanol 96% then vortexed and sonicated for 10 minutes, then centrifuged (repeat this procedure three times). The supernatant was added ethanol 96%, scanning the absorbance at a wavelength of 425 nm (Trinidad *et al.,* 2012) [41].

Standard Curve

Quercetin standard was weighed at 10 mg accurately, add 0.3 mL of sodium nitrite 5%, wait for 5 minutes, add 0.6 mL of aluminum nitrate 10%, wait 5 minutes, and add 2 mL of 1.0 M sodium hydroxide, add 10 mL, dilute according to the concentration to make a standard curve, and read the absorbance at 510 nm (Nguyen *et al.*, 2012). The standard curcumin was carefully weighing, put into a measuring flask, and adding ethanol 96% solvent. Standard curve dilution was made: 1000 mg/Kg (A), diluted up to 10 times, standard concentration 100 mg/ Kg (B), and a standard curve solution to make concentration series of solution B (Trinidad *et al.*, 2012) [41].

Compatibility Test of SNEDDS Formula

Herbal imunity extract was dissolved with each component of SNEDDS. The oils phase was tamanu oil, coconut oil, and candlenut oil. The selected surfactants were tween 80, and the co-surfactants phase were propylene glycol and polyethylene glycol 400. Extracts that can be mixed homogeneously in each oil-surfactant-co-surfactant were selected as SNEDDS constituent components (Ermawati *et al.*, 2020) [14].

SNEDDS Formula

SNEDDS preparation was formulated with various concentrations of surfactants. The SNEDDS formula consists of tamanu oil: tween 80: propylene glycol with a composition of 1: 7: 1, 1: 8: 1, and 1: 9: 1. The total weight of the SNEDDS was 5 grams, then the Herbal imunity extract was incorporated into the SNEDDS (Ermawati *et al.*, 2020) [14].

Drug Loading of SNEDDS

Herbal imunity extract of 100 mg, 200 mg, and 300 mg was incorporated into the SNEDDS formula that formed with different surfactant concentrations, respectively. Physical property tests were carried out, including transmittance percentage, emulsification time, stability test, and particle size analysis. The system's most significant dose that can be loaded and produces the smallest particle size is selected as the optimal system (Ermawati *et al.*, 2020) [14].

Physical Properties Test of SNEDDS

Transmittance Test. SNEDDS 100.0 µL was added to water in a 5 mL measuring flask and homogenized with a vortex for 60 seconds. The absorbance of the solution was measured at a maximum wavelength of 650 nm using UV-VIS spectrophotometry. Water was used as a blank. **Emulsification time**. SNEDDS of 200 µL was dissolved in 250.0 mL of AGF medium at 37 °C, stirred using a magnetic stirrer at 100 rpm. Observations were made on time required for SNEDDS to form an oil/water emulsion as indicated by the homogeneous dissolution of SNEDDS in AGF media. **Stability Test**. SNEDDS took 1.5 mL of each formula into eppendorf 2.0 mL, stored at 4 °C and 40 °C for 24 hours, respectively, at each temperature (the treatment was repeated for six cycles). SNEDDS was then centrifuged for 10 minutes at 6000 rpm. Calculate the difference between the separation height and the total height of the SNEDDS so that the F value was obtained (Ermawati *et al.*, 2020) [14]. **Particle size and PDI**. SNEDDS of 100 µL was diluted with AGF media in a 5.0 mL measuring flask, then 3.0 mL was taken and put into a cuvette for analysis using the HORIBA SZ-100 instrument. The particle size data obtained were the average particle size and polydisperse index.

TLC Analysis of SNEDDS's Optimum Formula

Curcumin standard solution of 100 ppm and extract solution of 100 ppm was spotted with a volume of 10 µL on the stationary phase of aluminum silica-Gel 60 F254. The mobile phase for flavonoid was n-hexan: ethyl acetate: methanol: water (65: 25: 10: 5 v/v). The mobile phase for curcumin was chloroform: dichloromethan (32.5: 67.5 v/v). The TLC plates were observed under visible and UV light at 254 nm and 366 nm. The Rf value was calculated as a comparison between the distance the solute eluted in line with the mobile phase eluted (Kautsari *et al.*, 2020). Spots on the stationary phase that was similar to standard curcumin spots and have the same Rf value indicate that the Herbal imunity extract contains curcumin.

Data Analysis

Statistical analysis used the IBM SPSS Statistics 21 program with the One Way ANOVA to analyze if there were significant differences in the effect of surfactants on the results of the SNEDDS physical tests and PSA with a significance value of $p<0.05$. The results to conclude that the selected SNEDDS formula for Herbal imunity extract met the requirements for a good SNEDDS preparation.

Result and Discussion

A plant determination test was conducted to determine the species of medicinal plants used in the study. Medicinal plant species determine the content of active metabolites in the plant, differentiating between medicinal plants in one genus. The results of the determination test were presented in Table 1.

Table 1: Determination results of Herbal imunity ingredients

Determination Results	Documents Number
Zingiber officinale Roscoe	026/UN27.9.6.4/Lab/2023
Curcuma longa L	027/UN27.9.6.4/Lab/2023
Centella asiatica (L.) Urb.	024/UN27.9.6.4/Lab/2023
Cinnamomum burmanni (Ness&T. Nees) BI.	025/UN27.9.6.4/Lab/2023

The yield of the viscous extract from the maceration process of the Herbal immunity components with ethanol 70% solvent was 108.10 grams with a yield value of 10.81% w/v. The moisture content in the extract was 22.74%. According to Voight (1994), there were several types of extract, namely dried extract with moisture content <10%, thick extract 5-30&, and liquid extract>30%. In this study, the kinds of extract were thick extract.

The results of the linear regression equation for the standard curcumin curve were $y=0.1668x + 0.0045$ with a linearity value (r) of 0.9999, and the levels of curcumin in Herbal immunity extract were 1.39±0.005% w/w. The results of the linear regression equation for the standard quercetin curve were $y=0.0032x + 0.0026$ with a linearity value (r) of 0.9992, and the levels of quercetin in Herbal immunity extract were 4.05±0.02% w/w. The content of flavonoid total was higher in ethanol 70% solvent because it has semi-polar properties when compared to curcumins. Previous research stated that the Total Flavonoid Content of water extract of 30.09 ± 2.67 mg QE/g was significantly higher than ethanolic extract of 23.03 ± 2.89 mg QE/g. Different

solvents had potential to extract curcumin and extraction with ethanol gave the highest yield (Popuri and Pagala, 2013) [27]. (Fig 1).

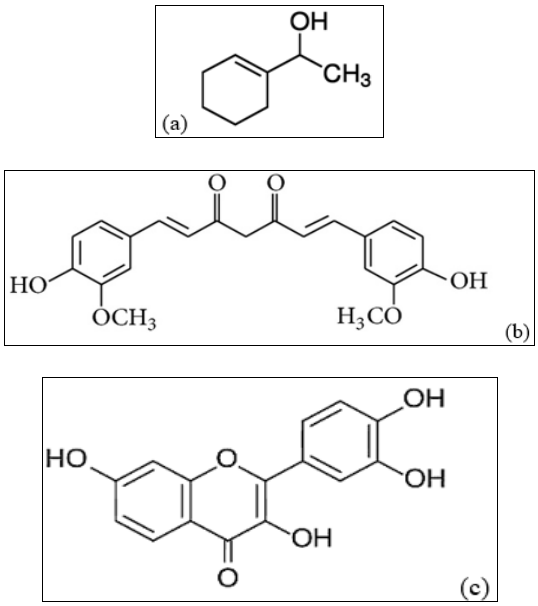


Fig 1: Molecular structure of ethanol 70% (a), curcumin (b), and flavonoids (c)

Results of the study revealed the presence of high concentration of flavonoids in *C. asiatica* leaf which include, naringin (4688.8 ± 69 µg/100 g), rutin (905.6 ± 123 µg/100 g), quercetin (3501.1 ± 107 µg/100 g) and catechin (915.87±6.01 µg/100 g) (Mohd Zainol *et al.,* 2009) [25]. The antioxidant activity of the C. *Asiatica* was correlated with total phenolic and flavonoid content with values IC_{50} achieving 2324.26 µg/mL in aqueous extract, and 1744.77 µg/mL in Ethanolic extract (Nguyen *et al.,* 2012). *Z. officinale* Rosc. and *C. longa* L. from Korea showed contents of curcumin (12.2 µg/mg) and polyphenols (85.7 µg/mg) (Jung *et al.,* 2012) [20]. Chemical constituents, especially flavonoids, from Indonesian cinnamon were successfully large-scale macerated in ethanol. Ethanol was selected as the best solvent for extraction according to the yield percentage, flavonoid content, and antioxidant activity in the preliminary solvent screening. The most excellent solvent to extract flavonoids was ethanol due to its high yield (21.50%), flavonoid content ($0.01749 \pm 8.0 \times 10^{-5}$ mg QE/g extract), and antioxidant activity (IC_{50} $0.0162 + 7.5 \times 10^{-4}$ mg/mL) (Rahayu *et al*., 2022) [31]. Based on the study results, it can be concluded that ethanol is a suitable solvent for extracting Herbal immunity, and the dominant active components contained in it are total flavonoids and total curcumin.

The vegetable oils are chosen because they are environmentally friendly, making them easier to degrade by microorganisms (Patel *et al.,* 2010) [26]. The three types of oil have long-chain triglycerides, which can increase drug transport through the lymphatic system to reduce first-pass metabolism. The surfactant component used is Tween 80; Tween 80 is used because it belongs to the non-ionic surfactant class and is relatively non-irritating and non-toxic (Rowe *et al.,* 2009) [34]. The co-surfactant components used are PEG 400 and propylene glycol. Both are short-chain alcohol groups that have a role in facilitating the mixing of water and oil (Azeem *et al.,* 2009) [5]. Based on the results of the compatibility test that has been carried out with homogeneity and stability parameters, the SNEDDS components of the Herbal immunity extract components selected were coconut oil, Tween 80 as a surfactant, and propylene glycol as a co-surfactant (Fig 2).

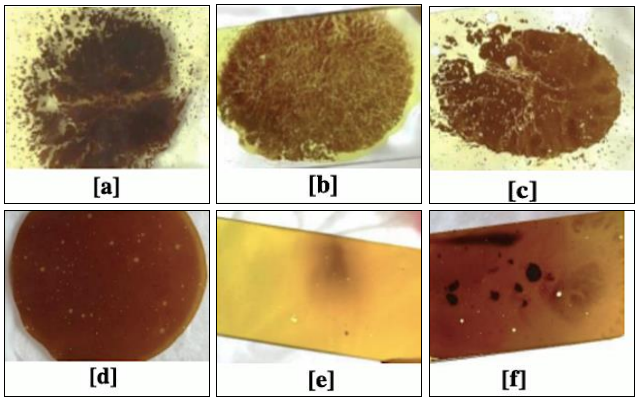


Fig 2: The results of compatibility test of Herbal immunity extract into SNEDDS components. Candlenut oil (a), Tamanu oil (b), Coconut oil (c), Tween 80 (d), Polyethylene glycol (e), propylene glycol (f)

Coconut oil belongs to the medium chain triglycerides containing 6-12 fatty acids and carbon. Triglycerides in coconut oil include caprylic acid, tamarind capric, and lauric acid (Chinwong *et al*., 2017) [8]. Surfactants with an HLB value of > 10 were hydrophilic and dissolved in water, and the type of the resulting emulsion was oil in water (o/w) (Banker *et al*., 2002; Debnath *et al*., 2011) [6, 10]. Tween 80 was a non-ionic surfactant group with an HLB value of 15 that can accelerate the formation of oil-in-water (o/w) emulsions, resistant to changes in pH, safe, biocompatible, and safe for oral preparations (Azeem *et al*., 2009) [5].

Table 2: The results of physical properties of Herbal immunity extract SNEDDS with various loading dose of extract

Loading Dose	SNEDDS Formula	Physical Properties Test		
		Transmittance (%)	Emulsification time (second)	F value by centrifugation (cm)
Herbal immunity extracts 100 mg	1:7:1	88.40±0.70	271±16.20	0.86±0.02
	1: 8:1	90.80±0.36	157±20.20	0.88±0.02
	1: 9:1	102.00±2.34 [a]	33±2.00	0.90±0.01[c]
Herbal immunity extracts 200 mg	1:7:1	82.86±0.41	271±16.20	0.83±0.02
	1: 8:1	83.06±0.15	157±20.20	0.85±0.02
	1: 9:1	42.30±0.36 [a]	34±3.00	0.87±0.02
Herbal immunity extracts 300 mg	1:7:1	91.56±0.20	241±15.30[b]	0.81±0.03[c]
	1: 8:1	85.26±0.35	125±7.00[b]	0.80±0.03
	1: 9:1	45.96±0.30 [a]	31±2.60[b]	0.79±0.02[c]

*SNEDDS weight was 5 grams; mean±SD; abc significant difference

Nanoemulsions can be considered nanometers in size if they have a transmittance percentage value of more than 90% (Pratiwi *et al.,* 2017) [28]. Visually transparent solutions have a transmittance value of almost 100%. Transmittance measurements were carried out at a wavelength of 650 nm because the maximum turbidity of the emulsion when the particle diameter is about 1.0 nm at a wavelength of 650 nm (Wang, 2014). According to Prihapsara *et al.* (2017) [29], an

emulsion can be a nanometer in size when it can transmit light at a wavelength of 650 nm. Based on the statistical analysis, a significance value of p > 0.05 was obtained. So, there is an influence between the surfactant concentration and the transmittance value. Increasing the extract weight will decrease the transmittance percent. The magnitude of the weight of the extract loaded in the nanoemulsion system can cause more excellent absorption of light passing through the system. So, the transmittance percentage value will be lower (Baloch *et al.*, 2019) [7]. Lower surfactant concentration in F1 and F2 compared to F3 resulted in lower oil binding ability, consequently, the longer it takes for the emulsion to form (Huda and Wahyuningsih, 2016) [18].

Emulsification time describes the time needed for the SNEDDS formula from the initial drop to be emulsified and form a homogeneous mixture in media with mild agitation. Media AGF (Artificial Gastric Fluid) is a liquid resembling gastric fluid with a pH of 1.2. AGF media will describe the mechanism of SNEDDS which is emulsified (oil in water) in gastric fluid (AGF) then the micelles in the intestinal fluid will enter the intestinal lymphatic vessels so that they will be absorbed into the systemic tract (Date *et al.*, 2010) [9]. The AGF media was conditioned at 37 °C and stirred using a magnetic stirrer at 100 rpm according to the conditions in the human stomach. Emulsification time of fewer than 60 seconds is included in the SNEDDS type A category. The statistical analysis results showed a significance value of p> 0.05. So, it can be stated that there is an influence between surfactant concentration and emulsification time. Emulsification time is classified into 4 categories: A, B, C, D, and E. Category A is an emulsion that can be formed in < 1 minute with a transparent appearance. Category B requires an emulsification time of 1-2 minutes to produce a transparent emulsion. Category C can form emulsions within 2 minutes with a less clear appearance. Category D can form an emulsion over 2 minutes with a cloudy oily appearance. Category E can form an emulsion in more than 2 minutes with large oil globules visible on the surface. Categories A, B, and C are recommended for the SNEDDS formula (Gautama and Singh, 2014) [15].

The centrifugation test evaluates the separation between the oil phase and the surfactant by damaging the absorbed emulsifier or surfactant layer. A stable emulsion requires a large centrifugal force to damage the surfactant layer. An F value close to 1.0 indicates that the emulsion is relatively stable. In the centrifugation method and the cycling test, the statistical analysis results showed a significance of p> 0.05, so it can be stated that there is an effect between surfactant concentration and the F value (stability). The higher surfactant concentration affects SNEDDS to become more stable.

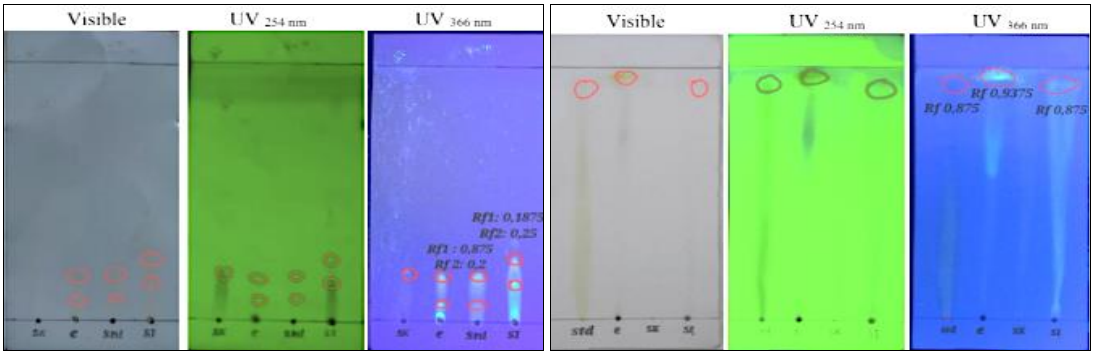
SNEDDS droplet size is an essential factor in self-emulsification formation because it will determine the speed and rate of drug release for absorption (Artanti *et al.*, 2021) [4]. Droplets can be considered nano-sized if they have a particle size between 10-100 nm (Artanti *et al.*, 2021) [4]. The droplet size distribution (PDI) is a parameter of the uniformity and reliability of the nanoemulsion preparation method. According to Date *et al.* (2010) [9] particle size and distribution are the most critical characteristics in nanoparticle systems because they estimate *in vivo* distribution, biology, toxicity, and targeting ability of nanoparticle systems. The polydispersity index, or particle size distribution, is a standard deviation value of the average particle size used as a parameter of uniformity and reliability of the nanoemulsion preparation method. The Polydispersity index shows the particle size distribution where the Polydispersity index range is between 0 and 1. A polydispersity index value close to 0 indicates a homogeneous or uniform distribution of particles, while a Polydispersity index value of more than 0.5 indicates a heterogeneous particle distribution.

Table 3: The results of paticle size analysis and PDI value of herbal immunity extract nanoemulsion

Loading Dose	SNEDDS Ratio	Paticle Size (nm)	Polydisperse Index
Herbal immunity extracts 100 mg	1:7:1	15.08±0.05	0.19±0.01
	1: 8:1	15.70±0.17	0.28±0.009
	1: 9:1	18.82±0.29	0.33±0.03
Herbal immunity extracts 300 mg	1:7:1	17.06±0.15	0.32±0.01
	1: 8:1	32.88±2.58	0.29±0.008
	1: 9:1	12.52±0.21*	1.00±0.0

*Significant different; mean±SD

Based on the results of the analysis, F1 with an extract loading of 300 mg selected as a formula that meets the requirements of nanoemulsion in this study with consideration of the ability to load the largest dose of extract and has the smallest particle size of 17.06 nm with a Polydispersity Index of 0.32 which indicates homogeneity and uniformity of the particle size distribution. The selected SNEDDS consists of coconut oil, tween 80, and propylene glycol in a ratio of 1:7:1. The test results for the characteristics of the selected formula include percentages transmittance of 42.26%, emulsification time of 241 seconds, and phase separation of 0.81. The test results almost did not meet the requirements on several parameters, including transmittance percentage < 90%, time emulsification > 120 seconds, and precipitate formation, indicating preparations needed to be more stable in hot and cold temperature storage. However thus, the selection of the most critical parameters in the SNEDDS was carried out by considering the results of the characteristic test based on the specific needs of the SNEDDS preparations, namely getting the smallest particle size with the largest extract dose.



[a] [b]

Fig 3: The results of TLC anlaysis of the choosen formula of Herbal immunity extract. [a] for curcumin and [b] for flavonoid total. Sk is standar, e is extract, S1 is SNEDDS extract F1 and Sk is SNEDDS without extract. The mobile phase of chloroform: dichloromethan (32.5: 67.5 v/v) was used to detect curcumin. The stationary phase is silica gel 60 F254. The mobile phase of n-hexan: ethyl acetate: methanol: water (65: 25: 10: 5 v/v). Spotting observations were made in visible light, UV 254, and UV 366

Based on previous research, the Rf value of the curcumin standard is 0.22 (Kautsari *et al*., 2020). Various solvents at different polarities were pre-tested in TLC to separate curcuminoids. Chloroform: methanol at 95:5 showed better resolution of Rf value at 0.75, 0.55, 0.27, as Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin, respectively (Revathy, Elumalay, and Anthony, 2011). The thin layer chromatography test results obtained spots on formula 1 of SNEDDS 300 mg, ethanolic extract, curcuminoids standard, and SNEDDS without extract. Fig 3 showed that the ethanol 70% extract of herbal immunity and curcumins standard have a spot match with Rf values of 0.088 and 0.20, respectively. The Formula 1 of SNEDDS 300 mg has two spots with an Rf value close to the curcuminoid standard of 0.19 and 0.25, respectively. Based on research by Kautsari *et al*. (2020), standardized curcuminoids resulted in 3 compound separations at an Rf1 value of 0.03; Rf2 0.08 and Rf3 0.22 identified as bisdemethoxycurcumin, demethoxycurcumin, and curcumin. This shows that in ethanol 70% extract of herbal immunity components and formula 1 of SNEDDS 300 mg contained compounds with characteristics that were similar to curcuminoids.

Quercetin is used to identify total flavonoid compounds because it belongs to the group of flavonoid compounds, which has five hydroxyl groups and can scavenge free radicals. It is also the most widely distributed compound in plants. In previous studies, the standard Rf value for quercetin was 0.80 (Khairunnisa *et al*., 2022) [22]. The thin layer chromatography for phenols using a methanolic extract of Centella in the solvent system gave a Retention factor (Rf) value of 0.83, similar to that of standard gallic acid (Desai *et al.*, 2013) [11]. Flavanoids showed their presence in all extracts with one spot in each (Rf 0.8 for acetone, 0.918 for methanol, 0.816 for chloroform, and 0.737 for aqueous extract) (Sonam, Singh, and Pooja, 2017) [39]. The Rf value of the extract and SNEDDS extract is close to the Rf value of the quercetin standard. Thin layer chromatography test results for compound detection Flavonoids showed that formula 1 of SNEDDS 300 mg and standard quercetin had spot similarities with an Rf value of 0.88. In the ethanol 70% extract of the herbal immunity component, there were spots fluoresce in a 366 nm UV lamp with an Rf value of 0.94 which was close to the Rf standard of flavonoids. This shows that the ethanol 70% extract of herbal immunity components and formula 1 of SNEDDS 300 mg contains compounds similar to quercetin.

Conclusion

Differences in surfactant concentrations affect particle size and polydispersity index values of SNEDDS of herbal immunity extract. The higher concentration of surfactant will decrease the transmittance percentage, delay the emulsification time, and potential to unstable of SNEDDS. The formula with a ratio of coconut oil: tween 80: propylene glycol (1: 7: 1) meets the requirements compared to other formulas. This formula can load extracts at a dose of 300 mg/5 grams of SNEDDS with a particle size of 17.06 nm and a PDI of 0.32. The total flavonoid content in herbal immunity extracts was 4.05% w/w and curcumin 1.39% w/w. After being formulated in SNEDDS, it still contained the active ingredients, total flavonoids, and curcumin.

References

1. Akbar ND, Nugroho AK, Martono S. By simplex lattice design and box behnken design, Jurnal Ilmiah Farmako Bahari. 2022; 13(1).
2. Anggoro D, Rezki RS, Siswarni MZ. Ekstraksi Multi Tahap Kurkumin dari Temulawak (Curcuma xanthorriza Roxb) Menggunakan Pelarut Etanol, Jurnal Teknik Kimia USU. 2015; 4(2):39-45.
3. Artanti AN, Prihapsara F, Ermawati DE, Shofa AS. Optimization of Self-Micro Emulsifying Drug Delivery System) for Soursop Leaf (Annona muricata Linn.) Chloroform Extract, Majalah Obat Tradisional. 2020; 25(2):81-89.
4. Artanti AN, Prihapsara F, Mas' ud N, Ermawati DE. Optimization of the Proportion of Surfactant, Co-Surfactant, and Candlenut Oil for Self-Nanoemulsifying Drug Delivery System (SNEDDS) of Secang Heartwood (Caesalpinia sappan L.) Methanolic Extract, ALCHEMY Jurnal Penelitian Kimia. 2021; 17(1):124-131.
5. Azeem A, Rizwan M, Ahmad FJ, Iqbal Z, Khar RK, Aqil M, Talegaonkar S. Nanoemulsion components screening and selection: A technical note, Aaps Pharmscitech. 2009; 10:69-76.
6. Banker GS, Siepmann J, Rhodes C. (Eds.). Modern pharmaceutics. CRC Press, 2002.
7. Baloch J, Sohail MF, Sarwar HS, Kiani MH, Khan GM, Jahan S, *et al*. Self-nanoemulsifying drug delivery system (SNEDDS) for improved oral bioavailability of

chlorpromazine: *In vitro* and *in vivo* evaluation. Medicina. 2019; 55(5):p210.
8. Chinwong S, Chinwong D, Mangklabruks A. Daily consumption of virgin coconut oil increases high-density lipoprotein cholesterol levels in healthy volunteers: A randomized crossover trial. Evidence-Based Complementary and Alternative Medicine, 2017.
9. Date AA, Desai N, Dixit R, Nagarsenker M. Self-nanoemulsifying drug delivery systems: Formulation insights, applications and advances, Nanomedicine. 2010; 5(10):1595-1616.
10. Debnath SUBHASHIS, Satayanarayana KV, Kumar GV. Nanoemulsion-a method to improve the solubility of lipophilic drugs. Pharmanest. 2011; 2(2-3):72-83.
11. Desai SS, Shruti K, Sana S, Sapna P, Zainab M, Swati H, *et al*. Phytochemical analysis, free radical scavenging and antioxidant profiling using chromatographic techniques for Centella asiatica. International Journal of Biotechnology and Bioengineering Research. 2013; 4(7):687-696.
12. Di L, Kerns E. Drug-like properties: Concepts, structure design and methods from ADME to toxicity optimization. Academic press, 2015.
13. Ermawati DE, Sasmito E, Mufrod M, ND PE, UA NP, Anggi KD, *et al*. Optimum Dose and Formulation of Centella asiatica L. Urban Extract Against IgG of Wistar Strain Male Mices which Induced by BCG Vaccine, Journal of Food and Pharmaceutical Sciences. 2016; 4(3).
14. Ermawati DE, Pambudi RK, Aviwiandari V, Farida Y, Rohmani S, Kundarto W, *et al*. Developing formula of SNEDDS (self-nano emulsifying drug delivery system) antihypertensive herbals "Hortus Medicus", In AIP Conference Proceedings. AIP Publishing LLC. 2020; 2237(1):p020062.
15. Gautam S, Singh AK. Self nanoemulsifying drug delivery system-a novalapproach for improving bioavailability. Journal of Drug Delivery and Therapeutics. 2014; 4(6):33-38.
16. Herman H, Murniati M. Inventarisasi Tanaman Obat Tradisional Untuk Penderita Diabetes Melitus Dan Hipertensi Di Desa Minanga Kecamatan Bambang Kabupaten Mamasa, Jurnal Farmasi Sandi Karsa. 2019; 5(1):26-32.
17. Hidayah I, Indradadi RB. Review artikel aktivitas imunomodulator beberapa tanaman dari suku Zingiberaceae, Jurnal Kesehatan Bakti Tunas Husada: Jurnal Ilmu-ilmu Keperawatan. Analis Kesehatan dan Farmasi. 2020; 20(2):181-193.
18. Huda N, Wahyuningsih I. Karakterisasi self-nanoemulsifying drug delivery system (SNEDDS) minyak buah merah (Pandanus conoideus Lam.). Jurnal Farmasi Dan Ilmu Kefarmasian Indonesia. 2016; 3(2):49-57.
19. Jafarzadeh A, Mohammadi-Kordkhayli M, Ahangar-Parvin R, Azizi V, Khoramdel-Azad H, Shamsizadeh A, *et al*. Ginger extracts influence the expression of IL-27 and IL-33 in the central nervous system in experimental autoimmune encephalomyelitis and ameliorates the clinical symptoms of disease, Journal of Neuroimmunology. 2014; 276(1-2):80-88.
20. Jung YS, Park SJ, Park JH, Jhee KH, Lee IS, Yang SA. Effects of ethanol extracts from Zingiber officinale Rosc. Curcuma longa L., and Curcuma aromatica Salisb. On acetylcholinesterase and antioxidant activities as well as GABA contents. Journal of the Korean Society of Food Science and Nutrition. 2012; 41(10):1395-1401.
21. Kautsari SN, Purwakusumah ED, Nurcholis W. Profil kromatografi lapis tipis ekstrak kunyit (curcuma longa linn) segar dan simplisia dengan variasi metode ekstraksi, Media Farmasi. 2021; 16(1):65-70.
22. Khairunnisa S, Hakim AR, Audina M. Perbandingan Kadar Flavonoid Total Berdasarkan Perbedaan Konsentrasi Pelarut Etanol Dari Ekstrak Daun Pegagan (Centella asiatica [L] Urban): Perbandingan Kadar Flavonoid Total Berdasarkan Perbedaan Konsentrasi Pelarut Etanol Dari Ekstrak Daun Pegagan (Centella asiatica [L] Urban), Journal Pharmaceutical Care and Sciences. 2022; 3(1):121-131.
23. Lesmana H, Alfianur A, Utami PA, Retnowati Y, Darni D. Pengobatan tradisional pada masyarakat tidung kota Tarakan: Study kualitatif kearifan lokal bidang Kesehatan, Medisains. 2018; 16(1):31-41.
24. Li M, Yue GGL, Tsui SKW, Fung KP, Bik-San Lau C. Turmeric extract, with absorbable curcumin, has potent anti-metastatic effect *in vitro* and *in vivo*, Phytomedicine. 2018; 46:131-141.
25. Mohd Zainol MK, Abdul-Hamid A, Abu Bakar F, Pak Dek S. Effect of different drying methods on the degradation of selected flavonoids in Centella asiatica. International Food Research Journal. 2009; 16(4):531-537.
26. Patel MJ, Patel NM, Patel RB, Patel RP. Formulation and evaluation of self-microemulsifying drug delivery system of lovastatin, Asian J Pharm Sci. 2010; 5(6):266-275.
27. Popuri AK, Pagala B. Extraction of curcumin from turmeric roots. Int J Innovative Res Stud. 2013; 2:289-299.
28. Pratiwi L, Fudholi A, Martien R, Pramono S. Self-nanoemulsifying Drug Delivery System (Snedds) for topical delivery of mangosteen peels (Garcinia mangostana L.,): Formulation design and *in vitro* studies, Journal of Young Pharmacists. 2017; 9(3):p341.
29. Prihapsara F, Harini M, Widiyani T, Artanti AN, Ani IL. Antidiabetic activity of self-nanoemulsifying drug delivery system from bay leaves (Eugenia polyantha Wight) ethyl acetate fraction. In IOP Conference Series: Materials Science and Engineering. IOP Publishing. 2017; 176(1):p012004.
30. Quyen NTC, Quyen NTN, Quy NN, Quan PM. Evaluation of total polyphenol content, total flavonoid content, and antioxidant activity of Centella asiatica. In IOP Conference Series: Materials Science and Engineering. IOP Publishing. 2020; 991(1):p012020.
31. Rahayu DUC, Hakim RA, Mawarni SA, Satriani AR. Indonesian Cinnamon (Cinnamomum burmannii): Extraction, Flavonoid Content, Antioxidant Activity, and Stability in the Presence of Ascorbic Acid. Cosmetics. 2022; 9(3):p57.
32. Revathy S, Elumalai S, Antony MB. Isolation, purification and identification of curcuminoids from turmeric (Curcuma longa L.) by column chromatography. Journal of Experimental sciences. 2011; 2(7).
33. Rohmah SAA, Muadifah A, Martha RD. Validasi

Metode Penetapan Kadar Pengawet Natrium Benzoat pada Sari Kedelai di Beberapa Kecamatan di Kabupaten Tulungagung Menggunakan Spektrofotometer Uv-Vis, Jurnal Sains dan Kesehatan (J. Sains Kes.). 2021; 3(2):120-127.
34. Rowe RC, Sheskey P, Quinn M. Handbook of pharmaceutical excipients. Libros Digitales-Pharmaceutical Press, 2009.
35. Saidah S, Kholifah K, Wahyuni L, Rahmatika AM, Ulfah M. Atraksi louis: Aktivitas kombinasi ekstrak sambiloto (andrographispaniculata) dan kayu manis (cinnamomumverum, sin. c. zeylanicum) terhadap sistem imun humoral (igg dan igm) pada mencit balb/c yang diinduksi vaksin hepatitis b, Jurnal Inovasi Teknik Kimia. 2017; 2(2).
36. Shofi M, Suwitasari F, Istiqomah N. Aktivitas antioksidan ekstrak etanol kamboja jepang (Adenium obesum) dan kamboja putih (Plumeria acuminata), Al-Kauniyah J Biol. 2020; 13(2):167-178.
37. Shoviantari F, Liziarmezilia Z, Bahing A, Agustina L. Uji aktivitas tonik rambut nanoemulsi minyak kemiri (Aleurites moluccana L.), Jurnal Farmasi dan Ilmu Kefarmasian Indonesia. 2019; 6(2):69-73.
38. Sogi DS, Sharma S, Oberoi DPS, Wani IA. Effect of extraction parameters on curcumin yield from turmeric. Journal of Food Science and Technology. 2010; 47:300-304.
39. Sonam M, Singh RP, Pooja S. Phytochemical screening and TLC profiling of various extracts of Reinwardtia indica. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(4):523-527.
40. Syafitri NE, Bintang M, Falah S. Kandungan fitokimia, total fenol, dan total flavonoid ekstrak buah harendong (Melastoma affine D. Don), Current Biochemistry. 2014; 1(3).
41. Trinidad TP, Sagum RS, de Leon MP, Mallillin AC, Borlagdan MP. Zingiber officinale and Curcuma longa as potential functional foods/ingredients. Food and Public Health. 2012; 2(2):1-4.
42. Widodo F, Lyrawati D, Suryana BPP. Potential of Topical Curcumin in Reduction of TNF-α expression and Synovium Hyperplasia on Wistar Rats of Rheumatoid Arthritis Model, Research Journal of Life Science. 2016; 3(1):40-48.
43. Yupitasari Y, Suryani D, Wahyudi A, Krisnasary A, Yunita Y. Gambaran Konsumsi Karbohidrat, Lemak, Natrium dan Kejadian Hipertensi pada Lansia di Panti Sosial Tresna Werdha Pagar Dewa Kota Bengkulu Tahun 2021 (Doctoral dissertation, Poltekkes Kemenkes Bengkulu), 2021.
44. Zulfa E, Novianto D, Setiawan D. Formulasi Nanoemulsi Natrium Diklofenak Dengan Variasi Kombinasi Tween 80 Dan Span 80: Kajian Karakteristik Fisik Sediaan, Media Farmasi Indonesia. 2019; 14(1):1471-1477.