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The Effect of Surfactant Concentration on Particle Size and Loading Dose of Immunity Jamu's Ethanolic Extract SNEDDS (Self-Nano Emulsifying Drugs Delivery System)

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ABSTRACT

Immunity jamu consists of ginger, turmeric, Centella, and cinnamon, that act as immunostimulant agents. However, the infusion is impractical and used a limited dose of the extract, so it is necessary to develop drug delivery to resolve that problem. The 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 SNEDDS to obtain the most loading dose but the smallest particle size. The formula consists of tamanu oil tween 80-propylene glycol of 1:7:1; 1:8:1; and 1:9:1 that incorporated extracts were 75, 150, and 375 mg. The physical tests included transmittance percentage, emulsification time on AGF media, phase separation, and stability test using the cycling test method. SNEDDS, then followed by the Particle Size Analyzer test. The results showed that the greater surfactant concentration produced a better transmittance value, a faster emulsification time, and stability. Formula with oil: surfactant: co-surfactant of 1:8:1 is a system that meets the requirement for immunity jamu with an optimal loading dose and small particle size compared to another formula. The extract's loading dose of 375 mg has a particle size of 27.17 nm and a polydispersity index of 0.25.
Keywords: Immunity jamu; immunostimulant; surfactant; SNEDDS; loading dose

INTRODUCTION

Jamu primarily consists of medicinal plants that Indonesian people 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). The Indonesian Law No. 36 of 2009 Act 59 states that traditional health services are based on the method of treatment, namely traditional health services with skills and ingredients (Lesmana *et al.,* 2018). The composition of Immunity Jamu is Ginjer, Turmeric, Centella, and Cinnamon, which are believed to maintain the body's stamina and immune system and prevent disease.

Ginger at a dose of 120 mg/Kg BW combined with turmeric can provide an immunostimulating effect because it can increase serum TNF- and IFN-. In contrast, a 300 mg/Kg BW dose can provide an immunosuppressant effect due to a decrease in IL-27 (Hidayah and Indradi, 2020). Research by Jafarzadeh *et al.* (2014) stated that an alcohol extract from ginger of 300 mg/Kg BW in mice provided immunosuppressant activity because it could significantly reduce serum IL-27 levels so that it could suppress inflammatory conditions. The research results by Widodo *et al.* (2016) showed that giving a curcumin dose of 110 mg/Kg BW can potentially improve abnormalities due to joint injuries. Meanwhile, according to Li *et al*. (2017), administering turmeric water extract at 200 mg/Kg BW in mice can increase immunity through T-cell simulation. *Centella asiatica (L.)* Urban has a high antioxidant activity of 84%. Administering Centella ethanolic extract at doses of 50 mg/Kg and 100 mg/Kg BW can improve the immune system, as indicated by increased IgG levels in the blood serum of mice induced by the BCG vaccine (Ermawati *et al.,* 2017). Cinnamon contains phytosterols which have a structure like cholesterol. Phytosterols can lower cholesterol levels by inhibiting the absorption of cholesterol in the intestines so that they can help reduce the amount of cholesterol that enters the bloodstream. The content of analgesic essential oils can stimulate blood circulation and relieve pain (Handayani and Paneo, 2021). Cinnamon acts as an enhancer of the IgM humoral immune system when combined with Sambiloto extract with a dose ratio of Sambiloto extract: Cinnamon extract of 300 mg: 150 mg and can increase the amount of IgG when combined with Sambiloto extract with a ratio of extract dose (Saidah *et al.,* 2017).

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Instant immunity jamu 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; 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). According to Date *et al*. (2010), 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). 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).

The components of the active ingredients of herbal medicine have different polarity properties, so an appropriate solvent is needed to extract the active metabolites in the herbal components so that it is optimal. Ethanol 96% solvent produces more yield than water solvent. This is in line with research conducted by Shofi *et al.* (2020) that the yield obtained from aqueous extracts was lower than using ethanol 96% because ethanol has a low polarity, thereby increasing the solvent's ability to attract active metabolite content in medicinal plants which are non-polar. While the water content in ethanol 96% is less than in ethanol 70%, its polarity is lower. Quercetin is a non-polar compound with low water solubility and is more soluble in organic solvents. According to Di and Kerns (2016), using higher ethanol concentrations in extraction can produce more quercetin levels. Differences in ethanol concentration can affect the yield value, total phenol, total flavonoids, and antioxidant activity in an extract. Then based on research conducted by Syafitri *et al.* (2014), the extract with ethanol 96% produces the highest of total flavonoids, while the extract with ethanol 70% produces the highest of total phenols. The polarity of the solvent influences these results, the higher the polarity of the solvent, the higher the phenol content. Meanwhile, the lower the polarity, the higher the total flavonoid compounds. Based on research conducted by Anggoro *et al.* (2015), extraction using ethanol 96% they have resulted in higher curcumin levels than ethanol 70%. The total Flavonoid content of Centella ethanolic extract is 23.03 ± 2.89 mg QE/g. The antioxidant activity of C. *Asiatica* was correlated with total phenolic and flavonoid content with values IC_{50} 1744.77 µg/mL in Ethanolic extract (Quyen *et al.,* 2020). Curcuma longa has significantly greater total polyphenols, flavonoids, anthocyanidins, and antioxidant activity (Trinidad *et al.,* 2012). Natural Curcumin belonged to Zingiber Officinale Roscoe and was known to possess natural odor, natural taste, natural color, and other pharmaceuticals (Jung *et al.,* 2012). Chemical constituents, especially flavonoids, from Indonesian cinnamon were successfully large-scale macerated in ethanol (Rahayu *et al.,* 2022).

Based on the formula optimization by Ermawati et al. (2020). 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), tamanu oil batch number 201103/177181 (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 96% (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 96% 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).

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, 2 mL of ethanol of 96% was added 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, centrifuged (repeat this procedure three times). The supernatant was added ethanol of 96%, scanning the absorbance at a wavelength of 425 nm (Trinidad *et al.,* 2012).

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 weighed, 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).

Compatibility Test of SNEDDS Formula

Immunity Jamu 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).

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 Immunity Jamu extract was incorporated into the SNEDDS (Ermawati *et al.,* 2020).

Drug Loading of SNEDDS

Immunity Jamu extract of 75 mg, 150 mg, 250 mg, and 375 mg was incorporated into the SNEDDS formula that formed with different

Table I. Determination results of Immunity Jamu 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

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).

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 the 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). **Particle size and PDI**. SNEDDS of 100 µL were 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 curcumin was chloroform: dichloromethane (32.5: 67.5 v/v). The mobile phase for flavonoid total was chloroform: methanol: water (65: 25: 4). The TLC plates were observed under visible and UV light at 254 nm and 366 nm. The Rf value is 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 Immunity Jamu 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 conclude that the selected SNEDDS formula for Immunity Jamu 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 I.

The yield of the viscous extract from the maceration process of the Immunity Jamu components with ethanol 96% solvent was 100.64 grams with a yield value of 10.06% w/v. The experimental value of curcumin yield ranged between 4.49 and 12.89% (Sogi *et al.,* 2010). The spectrophotometric method was chosen because it has advantages, including that it can be used to analyze many organic and inorganic substances, is selective, has a high degree of accuracy with an error percentage of 1 – 3%, is fast and precise, and can be used to determine minimal quantities of substances. Data results are accurate because the numbers read are printed directly in digital numbers or graphs that have been regressed (Rohmah *et al.,* 2021). 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 Immunity Jamu extract were 3.15% w/w. The

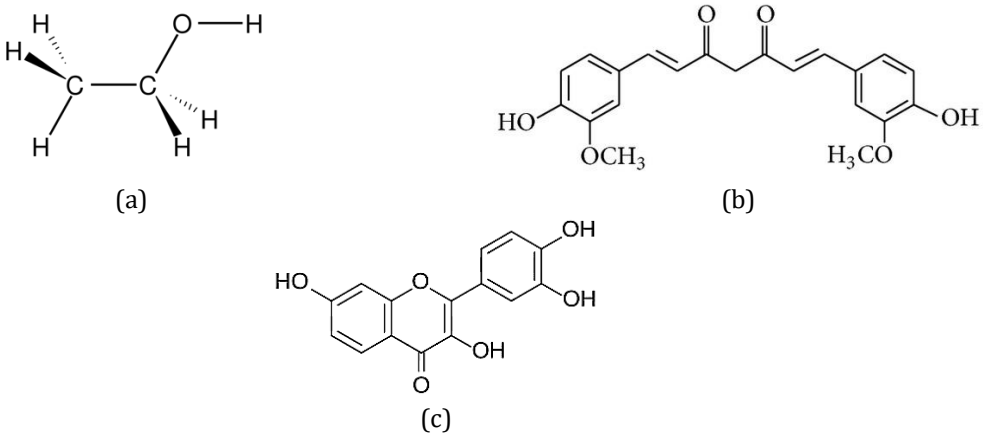

Figure 1. Molecular structure of ethanol 96% (a), curcumin (b), and flavonoids (c)

results of the linear regression equation for the standard quercetin curve are y=0.0032x + 0.0026 with a linearity value (r) of 0.9992, and the levels of quercetin in Immunity Jamu extract are 2.82% w/w. The content of curcumin is higher in ethanol 96% solvent because it has non-polar properties when compared to flavonoids. 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 the potential to extract curcumin and extraction with ethanol gave the highest yield (Popuri and Pagala, 2013). (Figure 1).

Results of the study revealed the presence of a 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). The antioxidant activity of 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). 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 ± 8.0 × 10^{-5} mg QE/g extract), and antioxidant activity (IC_{50} 0.0162 + 7.5 × 10^{-4} mg/mL) (Rahayu et al., 2022). Based on the study results, it can be concluded that ethanol is a suitable solvent for extracting Immunity Jamu, 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). 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). 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). Based on the results of the compatibility test that has been carried out with homogeneity and stability parameters, the SNEDDS components of the Immunity Jamu extract components selected are tamanu oil, Tween 80 as a surfactant, and propylene glycol as a co-surfactant (Figure 2).

Nanoemulsions can be considered nanometers in size if they have a transmittance percentage value of more than 90% (Pratiwi *et al.,* 2017). 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), an emulsion can be a nanometer in size

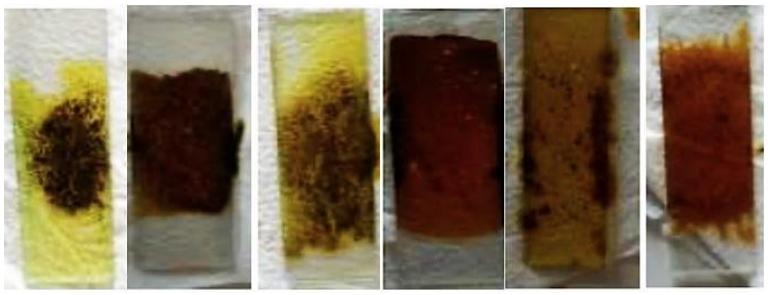

Figure 2. The results of the compatibility test of Immunity Jamu extract into SNEDDS components. Candlenut oil (1), Tamanu oil (2), Coconut oil (3), Tween 80 (4), Polyethylene glycol (5), propylene glycol (6)

Table II. The results of physical properties of Immunity Jamu extract SNEDDS with various loading dose of extract

SNEDDS Formula	Physical Properties Test			
	Transmittance (%)	Emulsification time (second)	F value by centrifugation (cm)	F value by cycling test (cm)
Without extract	94.00±2.34	50.56±7.70	1.00±0.00	1.00±0.00
Immunity Jamu extract 75 mg	92.55±3.89	25.42±2.75	0.88±0.04	0.91±0.01
Immunity Jamu extract 150 mg	81.95±4.34	22.99±3.55	0.88±0.06	0.88±0.02
Immunity Jamu extract 735 mg	53.97±7.27	30.88±0.58	0.90±0.06	0.77±0.02

***SNEDDS weight is 5 grams; mean±SD**

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.

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). 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.

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). Droplets can be considered nano-sized if they have a particle size between 10-100 nm (Artanti *et al.,* 2021). The droplet size distribution (PDI) is a parameter of the uniformity and reliability of the nanoemulsion

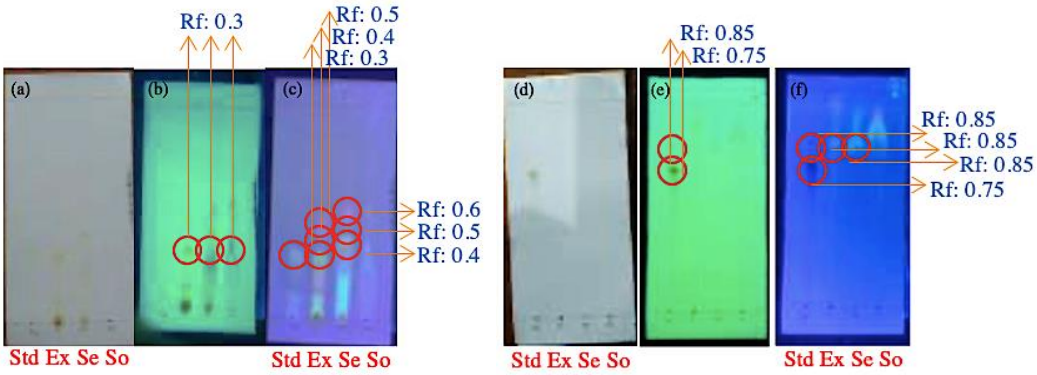

Figure 3. The results of TLC analysis of the chosen formula of Immunity Jamu extract. Std is standard, Ex is extract, Se is SNEDDS extract and So is SNEDDS without extract. The mobile phase of Chloroform: Dichloromethane (32.5: 67.5) was used to detect curcumin. The stationary phase is silica gel 60 F254 [a,b,c]. The mobile phase of Chloroform: Methanol: Water (65: 25: 4) was used to detect flavonoid total. The stationary phase is silica gel 60 F254 [d,e,f]. Spotting observations were made in visible light, UV 254, and UV 366.

preparation method. According to Date *et al.* (2010) 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. The results of SNEDDS PSA with a load of Immunity Jamu extract doses of 75 mg and 375 mg were 16.6±1.79 and 30.94±2.79, respectively. Meanwhile the PDI was 0.29±0.05 and 0.38±0.17, respectively. Based on the two SNEDDS formulas, the chosen formula is SNEDDS with the maximum dose incorporated of 375 mg because it has a particle size of 27.17 nm (20-200 nm).

The Rf value of curcumin Standard; extracts; SNEDDS extract 375 mg; and SNEDDS without extract under UV light 254 nm of 0.31; 0.30; 0.31, and 0.00, respectively. 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 Rf value of extracts under UV light 366 nm of 0.31; 0.40; and 0.51, respectively. The Rf value of SNEDDS extract 375 mg under UV light 366 nm of 0.41; 0.50; and 0.61, respectively. These results prove that the extract and SNEDDS extract 375 mg contain the curcuminoid active substance.

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. Quercetin standard Rf value; extract; SNEDDS extract 375 mg; and SNEDDS without extract of 0.75; 0.88, 0.85; 0.00, respectively. In previous studies, the standard Rf value for quercetin was 0.80 (Khairunnisa et al., 2022). 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). 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). The Rf value of the extract and SNEDDS extract is close to the Rf value of the quercetin standard.

CONCLUSION

Differences in surfactant concentrations affect particle size and polydispersity index values of SNEDDS of Immunity Jamu extract. The higher concentration of surfactant will increase the

transmittance percentage, speed up the emulsification time, and improve the stability of SNEDDS. The formula with a ratio of tamanu oil: tween 80: propylene glycol (1: 8: 1) meets the requirements compared to other formulas. This formula can load extracts at a dose of 375 mg/5 grams of SNEDDS with a particle size of 27.17 nm and a PDI of 0.25. The total flavonoid content in immunity jamu extracts was 2.82% w/w and curcumin 3.15% w/w. After being formulated in SNEDDS, it still contained the active ingredients, total flavonoids, and curcumin.

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