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Optimization of Selfnanoemulsifying Drug Delivery System (SNEDDS) Formulation of Ethil Acetate Fraction Soursop Leaf as Antioxidant

Dwi Saryanti*, Iwan Setiawan
Sekolah Tinggi Ilmu Kesehatan Nasional, Jl. Raya Solo - Baki, Bangorwo, Kwarasan, Kec. Grogol, Kabupaten Sukoharjo, Jawa Tengah 57552, Indonesia
*dwisaryanti@stikesnas.ac.id

ABSTRACT
Soursop leaves have acetogenin compounds that can be used to fight cancer in the presence of adenosine triphosphate (ATP) inhibition. Acetogenin is a long-chain fatty acid derivative (C23 or C34) which is very difficult to dissolve in water so that it will cause low oral bioavailability. The use of Selfnanoemulsifying Drug Delivery System (SNEDDS) as a drug delivery system can increase bioavailability and increase the solubility of a drug. Optimization of the use of VCO as the oil phase, tween 80 as surfactant and propylene glycol as co-surfactant in the SNEDDS formula using the Simplex Lattice Design method. The responses used in the optimization are % transmittance and emulsification time. Verification of the optimal SNEDDS formula obtained with one sample t-test. The optimal formula of SNEDDS ethyl acetate fraction of soursop leaves was tested for particle size with Partcle Size Analyzer, zeta potential with zeta sizer and antioxidant activity using ABTS. The optimal formula was obtained at the concentration of the components of VCO oil (0.56 ml), Surfactant (3.50 ml), and Propylene glycol (0.93 ml) with a desirability value of 0.769. The results of the verification of the optimal formula show that there is no significant difference between the prediction and the actual, which means that the Simplex Lattice Design method is valid and can describe the % transmittance response and emulsification time of SNEDDS ethyl acetate fraction of soursop leaves. The optimal formula for SNEDDS has an emulsification time of 53±1.527 seconds, transmittance value of % 96.3±0.360%. The particle size results obtained from the optimal formula were 13.83 nm ± 0.650, the polydispersity index was 0.143 ± 0.128. The zeta potential test results obtained were -0.23 mV ± 0.057. The optimal formula for the ethyl acetate fraction of soursop leaf SNEDDS has a very strong antioxidant activity with an IC50 value of 16.89 ppm.

Keywords: particle size; polydispersity index; SNEDDS; soursop; zeta potential

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INTRODUCTION

Cancer is a group of diseases characterized by the uncontrolled growth and development of abnormal cells (American Cancer Society, 2008). Cancer is one of the second deadliest diseases after cardiovascular disease. Based on data from the International Agency for Research on Cancer (IARC) in 2018 around 9.6 million people (nearly 10 million) died from cancer.One of the causes of cancer is free radicals. Free radicals are very reactive because they can cause chain reactions by attracting molecular electrons in the surrounding environment to complete their own orbitals (Marks et al., 2000). The speed of uncontrolled free radical formation in the body can cause oxidative stress.

The human body has a defense mechanism to fight oxidative stress due to free radicals by forming antioxidants (Pham-Huy et al., 2008). Antioxidants can delay or inhibit oxidation

reactions by free radicals. Antioxidants are immunomodulators so they can increase the strength of healthy cells to prevent the development of cancer cells. The mechanisms that have been successfully revealed are cytotoxicity (inhibition of cell division cycle) and induction of apoptosis.

Efforts to reduce the risk and prevention of cancer continue to be carried out through various studies. Researches are focused on the development of natural ingredients because one way to prevent cancer can use natural compounds that have anticancer activity. One of the plants that have anticancer activity is soursop leaf (Annona muricata L.). Soursop leaves contain acetogenin compounds that can be used to fight cancer by inhibiting adenosine triphosphate (ATP) (Widyaningrum et al, 2012). Acetogenin is a polyketide compound that has a structure of 30-32 unbranched carbon chains and is bound to a methyl-2-furanone group which has cytotoxic activity (Pradana et al, 2015).Asetogenin merupakan turunan asam lemak rantai Panjang (C23 atau C34) sehingga pada daun sirsak lebih banyak mengandung senyawa lipid dibandingkan pada bagian tanaman yang lain (Agu dan Okolie, 2017). Asitogenin merupakan senyawa yang sangat sulit larut dalam air sehingga akan menyebabkan bioavailabilitas oral yang rendah. Dalam penelitian Yang, dkk (2015) menunjukkan bahwa konsentrasi plasma maksimum dalam pemberian oral dengan dosis 100 mg/kg BB ekstrak etanol daun sirsak kurang dari 40 ng/mL dalam waktu kurang dari 1 jam.

The use of nanoparticles as a drug delivery system for anticancer therapy has great potential for cancer therapy in the future. The use of delivery systems for herbal medicines also aims to increase solubility, increase stability, protection from toxicity, increase pharmacological activity, gradual delivery and protection from physical and chemical degradation (Gupta et al., 2010; Goyal et al., 2011) .

Nanoemulsions are isotropic mixtures of oil, water, surfactants and stable and clear cosurfactant. Nanoemulsions can be formulated through SNEDDS (Self-Nanoemulsifying Drug Delivery System) which will form nanoemulsions spontaneously with mild agitation. SNEDDS Advantage is the ability to form nanoemulsions spontaneously in the gastrointestinal tract and the resulting droplet size is nanometer (Alwadei et al., 2019. Surfactants and cosurfactants in the nanoemulsion system work together to form a good and flexible interface system, and reduce the surface tension value to near zero so as to support the formation of stable nano-sized globules (Baloch, et al. , 2019; Apriani et al, 2018).

The SNEDDS formula consisted of VCO as the oil phase, tween 80 as the surfactant and propylene glycol as the cosurfactant. Tween 80 is a hydrophilic surfactant that has a Hydrophil Lipophil Balance (HLB) value of 15. The higher the addition of surfactant, the smaller the particle size, which is indicated by the percentage of neurotransmitters from the SNEDDS preparation of more than 90% (Diba et al, 2014). The use of propylene glycol as a cosurfactant can help surfactants reduce surface tension so that the resulting particle size will be smaller (Ermawati et al, 2020).

In the manufacture of SNEDDS, the oil phase of medium chain or long chain triglycerides can be used and have different degrees of saturation (Kuentz, 2011). The use of long-chain vegetable oils is a good choice in the preparation of SNEDDS and is often used even though the drug is highly lipophilic. In this study, VCO is used which is vegetable oil with a medium chain (medium long chain) (Ermawati, 2020). So based on these problems, a research was carried out on the manufacture of SNEDDS preparations from the ethyl acetate fraction of soursop leaves as an antioxidant.

METHOD

Blender (Cosmos), sieve No. 60, black cloth, baking dish, basin, wooden sotil, glass jar, measuring flask (asahi glasswere), beaker glass (pirex), stirring rod, metal spatula, gram scale, measuring pipette (Asahi), magnetic stirrer, uv-vs spectrophotometry (Shimadzu), cuvette, watch glass, porcelain cup (pyrex), pHmeter, thermometer, PSA (Horiba Scientific-100), Zeta sizer (Horiba Scientific-100), stove (Rinnai), measuring cup (Asahi), micropipette (TopPette Pipettor). Soursop leaves were obtained from Sawahan village, Ngemplak, Boyolali. Other ingredients obtained from the Agung Jaya shop are 70% ethanol (copy of chemistry), and from the chemical copyright shop are ethyl acetate (Bractaco), VCO oil, propylene glycol (Brataco), tween 80, aquadest, and NaCl, 37% HCl, MgCl, CaCl2, KCl, NaHCO3.

RESULTS

1. Soursop Leaf Extract

Soursop leaves were used as a sample in this study. Soursop leaves are dried and pollinated with the aim of increasing the surface area of the particles in contact with the solvent so that the extraction can take place effectively.

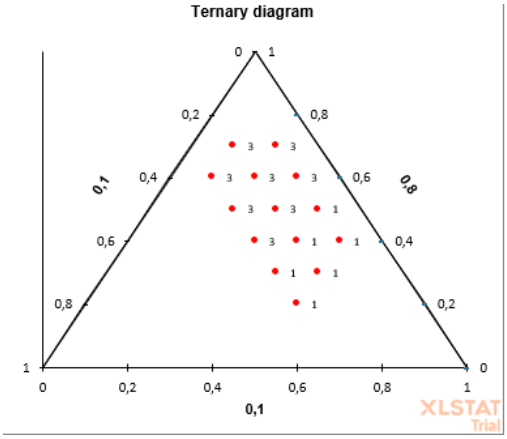


Figure 1. Triple plot diagram of oil phase, surfactant and co surfactant

Based on the phase diagram obtained from the oil phase, surfactants and cosurfactants were used to determine the range of use of these components in the preparation of the ethyl acetate fraction of soursop leaves. The results obtained were VCO was used in the 10%-20% range, tween 80 was used in the 70%-80% range and propylene glycol was used in the 10%-20% range.

2. Clarity test results

In the % transmittance test produced, it is said to be good if the results obtained are close to 100%, it can be said that the SNEDDS preparations made have a good level of clarity.

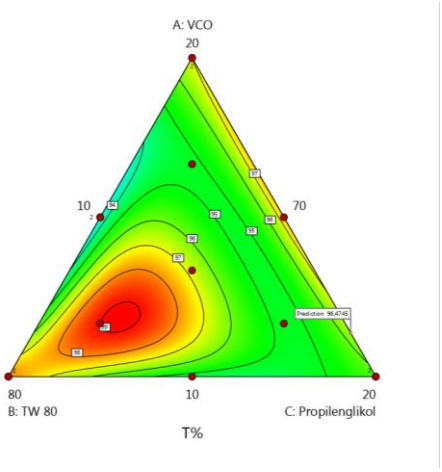


Figure 2. Contour plot of clarity test results

Based on Figure 2. The contour plot of the clarity test results can be seen in the blue area indicating that there is no effect on the transmittance value. The green area has a slight effect, while the yellow color indicates an increase in the transmittance value, but the red area has the most influence on the % transmittance value, the most dominant red color is in the tween 80 area. It can be concluded that tween 80 can increase transmitarn value in the SNEDDS formula.

Emulsification time test results Emulsion time test was carried out using artificial gastric fluid media.

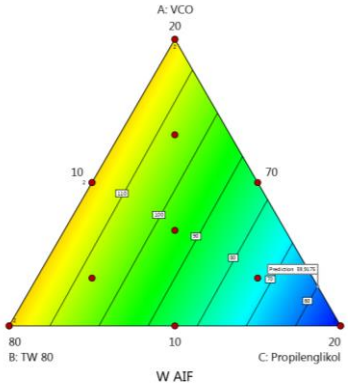


Figure 3. Contour plot of emulsion time test results

Based on Figure 3. Contour plot, the results of the emulsification time test show that the blue contour plot area has the fastest emulsion time, followed by green, yellow and red which have the slowest emulsion time.

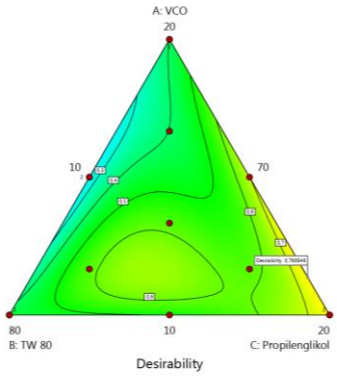
3. Optimization of Soursop Leaf Ethyl Acetate Fraction Optimal Formula

The optimal formula was selected using Design Expert 13.0 software with the simplex lattice design method, the parameters used to determine the optimal formula were % transmittance and emulsification time.

Table 1.
Optimization of the SNEDDS Formula for Soursop Leaf Ethyl Acetate Fraction

Parameter	Goal	Kriteria	Satuan	Importance
% T	*maximum*	91,2-99,1	%	+++
Waktu emulsi	*minimum*	50-136	detik	+++

The optimal formula for the ethyl acetate fraction SNEDDS obtained is based on Figure 4 with a composition of VCO 11.37%, Tween 80 70% and propylene glycol 18.62% with a



desirability value of 0.769.

Figure 4. Contour plot of the optimal formula SNEDDS

4. Verification of the Optimum Formula of the Ethyl Acetate Fraction of Soursop Leaf Extract

Table 2.
Verification of the SNEDDS Formula for Soursop Leaf Ethyl Acetate Fraction

Parameter	Prediksi SLD	Hasil uji	Signifikasi	interpretasi
% T	96.473	96,300±0,360	0.493	No significant differences
Emulsification time	59.896	53,666±1,527	0.019	Significantly differs

If it is seen from the test results obtained from the SNEDDS formula that is made it still meets the existing requirements, this can be seen in the clarity test results, which are close to 100% and the clarity time test results are less than 1 minute. It can be concluded that the results of using the simplex lattice design method are valid and can describe the physical properties of the soursop leaf ethyl acetate fraction SNEDDS in the clarity test and emulsification time.

5. Result of Determination of Particle Size Optimal Formula SNEDDS Ethyl Acetate Fraction

The average particle size obtained from the optimal formula was 13.83 nm ± 0.650. Particle size determines the rate of drug release so that it can affect drug absorption. The polydispersity index in the formula is 0.143 ± 0.128 (< 0.5) the acceptable range of polydispersity index is 0-0.5.

6. Results of Determination of Zeta Potential Optimal Formula SNEDDS Ethyl Acetate Fraction

The results of the zeta potential test carried out on the ethyl acetate fraction SNEDDS formula were -0.23 Mv ± 0.057. The stable zeta potential value is ± 30mV, but the minimum

acceptable zeta potential value is ± 20mV. Test Results of SNEDDS Antioxidant Activity of Soursop Leaf Ethyl Acetate Fraction with ABTS

SNEDDS can capture free radicals by 50%, so that there is a change from blue color until the color intensity decreases and the higher the sample concentration, the absorbance in the sample also decreases. The results of measurements of absorbance, % inhibition, and IC50 values on SNEDDS of the ethyl acetate fraction of soursop leaves are shown in table 3.

Table 3.
Test results of SNEDDS antioxidant activity of Soursop Leaf Ethyl Acetate Fraction

Konsentrasi	ABS	%inhibisi	Persamaan	IC50
5	0,6089	21,7955	$y = 2.3362x + 10.548$ $R^2 = 0.9978$	16,89
10	0,5174	33,5473		
15	0,4117	47,1230		
20	0,3345	57,0382		
25	0,2456	68,4562		

The results of the IC50 value obtained by SNEDDS of the ethyl acetate fraction of soursop leaves were 16.89 ppm. This IC50 value is included in the category of very strong antioxidants.

DISCUSSION

Soursop leaves were used as a sample in this study. Soursop leaves are dried and pollinated with the aim of increasing the surface area of the particles in contact with the solvent so that the extraction can take place effectively. The smaller the particle size, the greater the contact area between the sample and the solvent and the maximum amount of substance that will be extracted (Bernasconi, 1995).

The extraction method used is the maceration method because this method is simple and does not require special tools so it is easy to do. The yield obtained from this extraction was 30.47%. The results obtained are higher than the results of the research by Surbakti and Nadiya (2019) which yielded a yield of 6.57%. The difference in yield is due to the difference in the length of time the maceration is carried out, namely in this study it was carried out for 5 days but no remaceration was carried out, while in this study maceration was carried out for 5 days with remaceration.

In the liquid-liquid fractionation method, there is a distribution of a solute (solute) between two immiscible solvents. The solute will be distributed into both solvents after shaking and separation occurs (Ersita and Kardewi, 2016). The solvent used in the fractionation is ethyl acetate, because ethyl acetate has a lower polarity than water so it is expected to attract the active compounds contained in soursop leaves. Based on research by Ersita and Kardewi (2016), fractionation with ethyl acetate solvent resulted in a higher yield than the solvent N-hexane and methanol-water. The yield obtained from the fractionation results from this study was 10.33%.Hasil Uji Kelarutan Fraksi Etil Asetat Daun Sirsak

Based on the results of the solubility test, it was obtained that the highest solubility was in the oil phase of VCO, surfactant tween 80 and co-surfactant propylene glycol. Tween 80 is widely used as a surfactant in the SNEDDS system because it is a non-ionic surfactant that is safe to use orally and has lower toxicity than ionic surfactants (Pouton and Porter, 2008).

The desirability value that is close to 1 indicates the ability of a program to produce products that meet the requirements based on predetermined criteria, so that the closer the desirability value is to 1, the more perfect the resulting formula, because the optimum formula is the formula with the maximum desirability value (Ramadhani et al. , 2017).

The average particle size obtained from the optimal formula was 13.83 nm ± 0.650. Particle size determines the rate of drug release so that it can affect drug absorption. The polydispersity index in the formula is 0.143 ± 0.128 (< 0.5) the acceptable range of polydispersity index is 0-0.5. The low polydispersity index value indicates that the formula that has been made by the dispersion system can be more stable in the long term (Adi, et al., 2019).

The results of the zeta potential test carried out on the ethyl acetate fraction SNEDDS formula were -0.23 Mv ± 0.057. The stable zeta potential value is ± 30mV, but the minimum acceptable zeta potential value is ± 20mV. The negative value in the test results shows the charge of the zeta potential, the value of the charge formed can be influenced by the constituent components and the dispersing medium used. (Adi, et al., 2019).

CONCLUSION

The optimal formula of SNEDDS ethyl acetate fraction of soursop leaves obtained from the Simplex Lattice Design method with the composition of VCO oil (0.56 ml), Surfactant (3.50 ml), and Propylene glycol (0.93 ml) with a desirability value of 0.769. The optimal formula of SNEDDS acetate fraction of soursop leaves produced has a particle size of 13.83 nm ± 0.650, polydispersity index 0.143 ± 0.128. The zeta potential test results obtained were -0.23 mV ± 0.057 and had very strong antioxidant activity with an IC50 value of 16.89 ppm.

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