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Research Article

Self-Nanoemulsifying Drug Delivery System (SNEDDS) with Enhanched Solubilization of Ethanol Extract from Mangosteen Peels (Garcinia Mangostana, L.) for Treatment of Topical Gangrene Foot: Design and Optimization

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ABSTRACT
The aim of the present study is to develop and optimize self-nanoemulsifying drug delivery systems (SNEDDS) to improve the topical bioavailability of poorly soluble ethanol extract of mangosteen peels and to get optimum method of SNEDDS by simplex lattice design, using Design Expert software ®version 7. Solubility of ethanol extract of the mangosteen peels was estimated in various compositions to select proper components combinations. Virgin coconut oil/ VCO (oil), Tween® 80 (surfactants) as well as polietilenglikol 400 (PEG 400) (co-surfactants) were employed to construct pseudo-ternary phase diagrams. Transmittance and pH, droplet size, zeta potential, and thermodynamic stability were performed to optimize formulations from phase diagram. Fourteen formulations composed of VCO, Tween 80 and PEG 400 at simplex lattice design ratios were selected. The results showed that the ethanol extract of the mangosteen peels SNEDDS optimum consisting of Cremophor EL as the surfactant, PEG 400 as the co-surfactant, and VCO as the oil phase with a ratio of 5.27: 1: 1.72. Evaluation of SNEDDS with an optimum formulation with drug loading value of 125 mg/5 mL, emulsification time of 5,2 seconds, transmittance value of 74,6552 %, pH value 5,85 and has a particle size of 18,9 nm. Ethanol extract of the mangosteen peels loaded SNEDDS, with enhanced solubilization and nanosizing, and has potential to improve the absorption of drug and increase its topical antimicrobial activity against Staphylococcus aureus.

Keywords: Ethanol extract, mangosteen peels, SNEDDS, Simplex lattice design.

INTRODUCTION

Diabetes mellitus (DM) is a degenerative disease causing continually increasing insulin concentration which caused by genetics and lifestyle. High Blood Glucose Levels can make fatal complications such as gangrene foot, heart disease, kidney, blindness, and atherosclerosis. Diabetic gangrene in Indonesia occupies all 10 patients DM complications encountered that at the 2010 Year Period Patients at 4.6% of the population time period age 20 years until 79 Year[1].

Use of antibiotic treatment of diabetic wounds is very susceptible to resistance. In the last few years, many research leads of the use traditional medicine for the review can be pressed incident resistance. Mangosteen is planted traditionally used as a medicine. Many mangosteen peels have researched and are known to have the content of phenolic compounds, such as tannins, flavonoids and xanthone[2]. In the system development technology based pharmaceutical drug required a form that can increase the ability of active compounds for review penetrate, self-nanoemulsifying drug delivery system self (SNEDDS). SNEDDS allows the large scale manufacture to make an easy and economical manufacturing process that becomes the main attraction in the industry, as well as thermodynamically stable to facilitate storage[3]. SNEDDS is performed using the optimum formulation applying simplex lattice design with various concentrations of oil, surfactant, and co-surfactant. SNEDDS components are oils, surfactants, and co-surfactants. The oil used is VCO, oil coming from the main components inside in which lauric acid is a saturated fatty acid. Tween 80 as a surfactant and PEG 400 as a co-surfactant are compounds that have an affinity for water and oil phases.

MATERIALS AND METHODS

Reagents and chemicals

The fully-ripe fruits (dark purple peels) which selected for the study were freshly taken from Central Java, Indonesia. The other materials are standard α-mangostin (Sigma-Aldrich 98%), ethanol 70% (Dwicentra), methanol (Merck), aquades (Dwicentra), VCO (Bagoes), PEG 400 (Bratachem), Tween 80 (Bratachem), Tween 20 (Bratachem), Span 20 (Bratachem), Cremophore EL (Sigma Aldrich), Propilenglikol (Bratachem).

Instrumen condition

UV-Vis Spectrophotometer (Shimadzu) with quartz cell, FS 10 mm, Particle size analyzer (PSA) (Beckman coulter).

Preparation of plant extract

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Table 1: Design formulations from Design Expert.

Formula	Cremophore EL	PEG 400	VCO
F1	1.00	6.00	1.00
F2	3.50	3.50	1.00
F3	3.50	3.50	1.00
F4	4.33	1.83	1.83
F5	3.50	1.00	3.50
F6	1.00	1.00	6.00
F7	6.00	1.00	1.00
F8	1.00	6.00	1.00
F9	1.83	4.33	1.83
F10	2.67	2.67	2.67
F11	1.00	1.00	6.00
F12	1.00	3.50	3.50
F13	1.83	1.83	4.33
F14	6.00	1.00	1.00

Table 2: Solubility of ethanol extract in oil, surfactant and co-surfactant (mg / 10 mL).

Sample	Sample content of Compound (mg / 10 mL)
Olive oil	3,30
VCO	9,51*
Cremophore EL	6,16*
Tween 80	1,64
Span 20	5,43
Tween 20	4,74
PEG 400	6,44*
Propilenglikol	3,36

*Note: *) high dissolving ability*

Table 3: Transmittance analysis of SNEDDS formulation.

Formula	Cremophore EL	PEG 400	VCO	Transmittance (%)
F1	1.00	6.00	1.00	26.71
F2	3.50	3.50	1.00	41.83
F3	3.50	3.50	1.00	45.71
F4	4.33	1.83	1.83	64.56
F5	3.50	1.00	3.50	74.06
F6	1.00	1.00	6.00	57.58
F7	6.00	1.00	1.00	75.62
F8	1.00	6.00	1.00	23.91
F9	1.83	4.33	1.83	65.85
F10	2.67	2.67	2.67	57.83
F11	1.00	1.00	6.00	40.51
F12	1.00	3.50	3.50	48.98
F13	1.83	1.83	4.33	54.71
F14	6.00	1.00	1.00	73.27

One kg of powdered mangosteen peels was extracted via maceration for 72 hrs using ethanol 70%. Extracted samples were evaporated using waterbath until they become thick extracts.

Extract solubility measurements in oils, surfactants, and co-surfactants

A total of 10 mg of ethanol extract of the mangosteen peels were added to 10 ml VCO and olive oil as the oil phase,

Table 4: pH evaluation of SNEDDS formulation.

Formula	Cremophore EL	PEG 400	VCO	pH
F1	1.00	6.00	1.00	5.82
F2	3.50	3.50	1.00	5.25
F3	3.50	3.50	1.00	5.08
F4	4.33	1.83	1.83	5.62
F5	3.50	1.00	3.50	6.55
F6	1.00	1.00	6.00	7.46
F7	6.00	1.00	1.00	5.53
F8	1.00	6.00	1.00	5.77
F9	1.83	4.33	1.83	5.43
F10	2.67	2.67	2.67	6.25
F11	1.00	1.00	6.00	7.31
F12	1.00	3.50	3.50	6.68
F13	1.83	1.83	4.33	6.95
F14	6.00	1.00	1.00	5.71

Tween 80, Tween 20, Span 20, Cremophor EL as a surfactant, and PEG 400, Propylenglycol as co-surfactant. This mixture was conditioned in a water bath at 40° C for 10 minutes. The process of dissolving the fraction in a carrier was maximized by a sonicator for 15 minutes and left for two days at room temperature. After two days, insoluble part then separated by centrifugation at 3000 rpm for 20 minutes. Remaining samples of sediment were separated from the supernatant, extracted with 10 ml of methanol and its concentration was measured by spectrophotometry at a wavelength maximum of mangosteen peels. Samples that were able to dissolve more of the ethanol extract mangosteen peels were selected sample used for subsequent optimization phase.

Construction of pseudo-ternary phase diagrams

Determination the optimum formulation was conducted using simplex lattice design by Design Expert software ®version 7. Simplex lattice design optimized the mixture of three components which are oil, surfactant, and co-surfactant from 14 formulations in various compositions. SNEDDS characteristic of physical properties that were used in the determination of the optimum formulation is to test the transmittance and pH.

Transmittance study

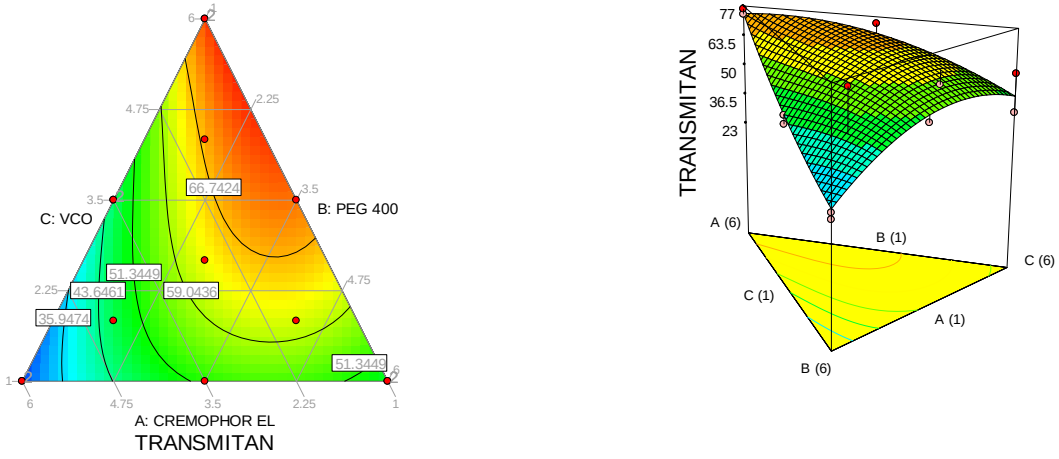
SNEDDS (100.0 µl) candidate preconcentrate formula with distilled water until a final volume of 5.0 mL. The mixture was homogenized with the aid of a vortex for 30 seconds. Emulsions that have been obtained then measured its absorbance at a wavelength of 650 nm [4].

pH

SNEDDS (100 µL) was dissolved in 5 mL of distilled water. Nanoemulsion measured PH for optimum formulation evaluation.

Drug loading study

Samples selected from the mangosteen peels (5, 10, 15, 20, 25.30, 50, 75, 100, 125.150 mg) were added to 5 mL SNEDDS optimum formulation. This referred to the way of making a solid dispersion method technique (Patel et al., 2012). SNEDDS was then homogenized with a vortex for 5 minutes, with a sonicator for 5 min, 45 ° C water bath for 5 minutes, this was repeated with a vortex for 5 minutes and sonicator for 10 minutes and conditioned in a 45 ºC



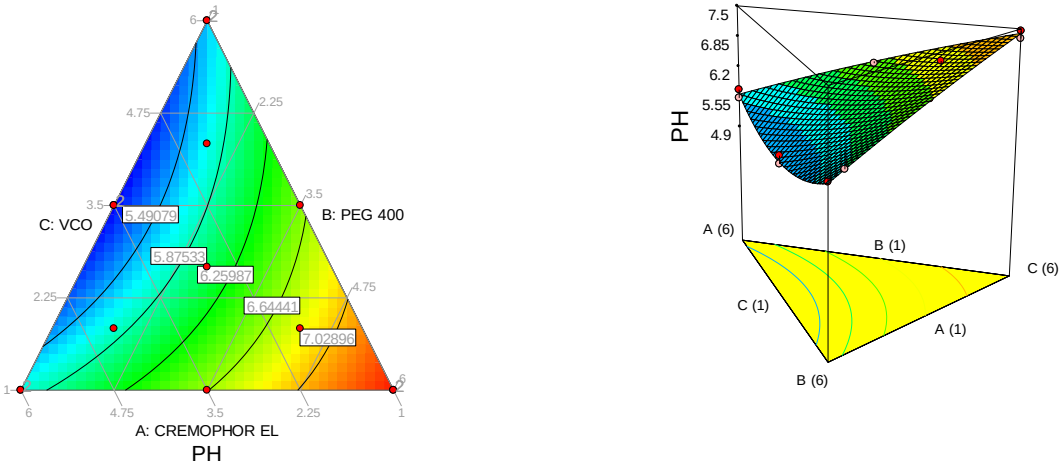
A B

Figure 1: (A) Countor plot response of transmittance, (B) 3D Surface response of transmittance

Based on data from transmittance and reflectance contour plot, then the equation simplex lattice design as follows:

Y = 11,60 A + 1,71 B+3,33 C-1,84 (A)(B) + 0,43 (A)(C)+1,24 (B)(C) +1,03 (A)(B)(C)

Note: Y = Transmittance, A = Cremophore EL, B = PEG 400, C = VCO.



A B

Figure 2: (A) Countor plot response of pH, (B) 3D Surface response of pH

Equation simplex lattice design for the pH response can be seen in the following equation:

Y = 0,72 A+0,76 B+0,98 C-0,09 (A)(B)+0,01 (A)(C)+ 6,99 (B)(C) + 2,31 (A)(B)(C).

Table 5: Numerical optimization with Simplex Lattice Design.

Name	Goal	Lower Limit	Upper Limit	Importance
Cremophore EL	Is in range	1	6	3
PEG 400	Is in range	1	6	3
VCO	Is in range	1	6	3
Transmitan	maximize	68	75	3
pH	Is in range	4.5	6.5	3

waterbath for 5 minutes. Observations on the solubility of the sample in SNEDDS were done visually.

Emulsification time measurement

It was 500 mL of distilled water which was conditioned on the top stirrer at 120 rpm. A total of 1 mL SNEDDS optimum containing active ingredients was dripped into the media quickly. Observations were made of the time required by nanoemulsion to form a homogeneous mixture which has been characterized by perfectly mixing SNEDDS in the media[5].

Droplet size analysis and zeta potential measurement

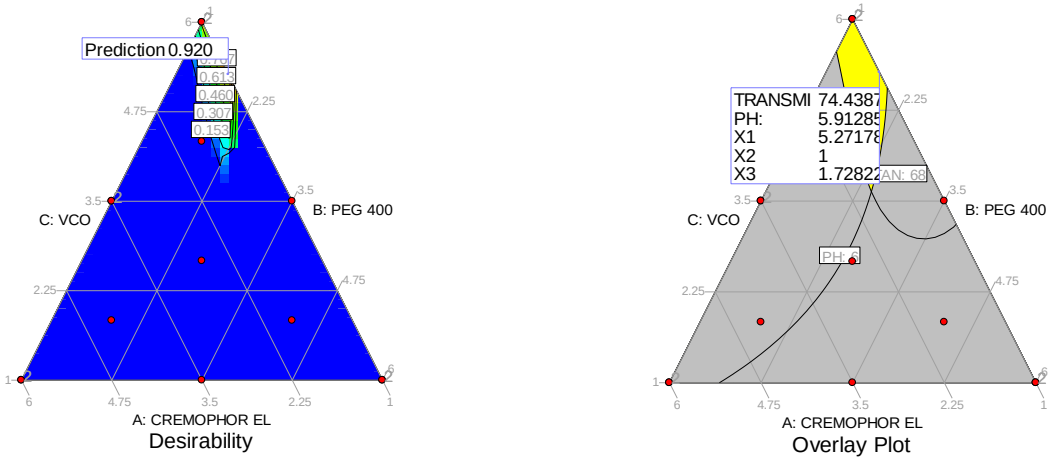
Measurings performed using a Particle Size Analyzer (PSA) were used to determine the size and distribution of nanoparticles. A total of 1 mL SNEDDS was mixed with distilled water to 5 mL, homogenized by way of flipping over. Furthermore, it was put in a cuvette for analysis.

Stability study

SNEDDS remained heated and maintained at 37ºC and homogenized by vortex for 30 seconds. It was observed every hour for 4 hours to determine its stability.

Table 6: Optimization Simplex Lattice Design with software design expert.

Number	Cremophore EL	PEG 400	VCO	Transmittance	pH	Desirability	Note
1	5.27	1.00	1.73	74.44	5.91	0.92	Selected
2	6.00	1.00	1.00	73.66	5.61	0.81	



A B

Figure 3: (A) Countor plot of desirability, (B) Countor plot of optimum formulation.

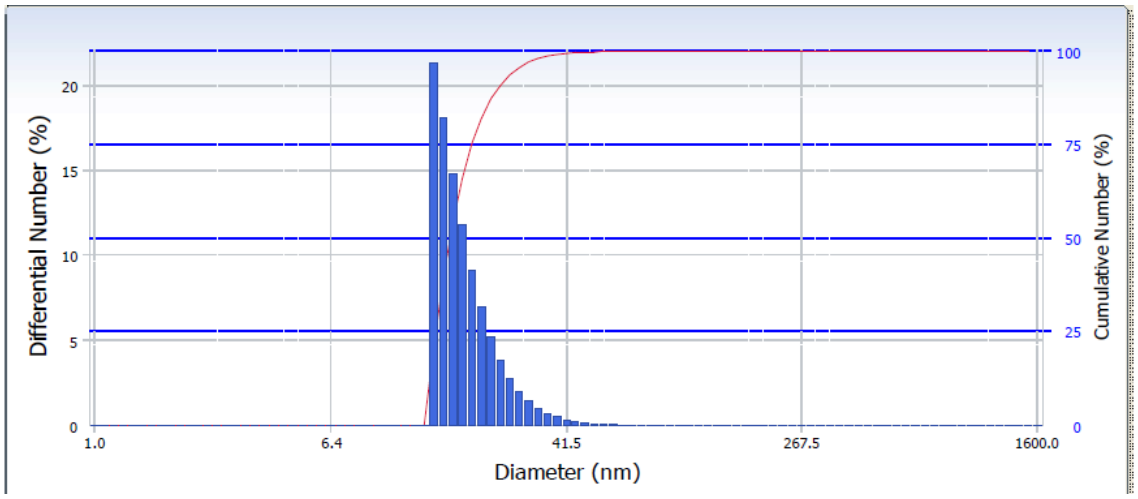


Figure 4: Particle size analysis.

Nanoemulsion is stable if it did not form lumps or sediment. SNEDDS were stored for three months.

RESULTS AND DISCUSSION

Screening oils, surfactants, and co-surfactants

Selection of oils phases based on oil capable of dissolving the components of the active compounds highest. Tests using two types of oil that are VCO and olive oil.

VCO capable of dissolving the highest extract due to its natural semipolar traits which easy to get into the oil phase. Drug solubility in oil of nanoemulsion is the most important factor because it is associated with the ability of nanoemulsion to keep the drug in dissolved form which is highly influenced by the drug solubility in the oil phase[6]. Mechanism action of surfactants is by lowering the interfacial tension between the oil phase and the aqueous phase after SNEDDS concentrates are dispersed in a dispersing medium to nanoemulsion. The type and amount of surfactants will affect the size of the oil droplets in the water phase. Cremophor EL as surfactant selected because it has the highest ability to dissolve the ethanol extract of mangosteen peels. Cremophor EL consists of hydrophobic constituents as much as 83% and 17% hydrophilic. Cremophor EL is soluble in ethanol, fatty acids, water, chloroform, and vegetable oils[7].

Co-surfactant formulations required to connect the surfactants molecules and helps the formation of increasingly smaller interfacial tension[8]. Co-surfactant molecules between the surfactant molecules on the surface of the oil globules will form oil globules with a sealed surface by surfactant molecules and co-surfactant.

Transmittance analysis

Transmittance indicates that clarity is one of the characteristic properties of the emulsion that need to be measured because it can influence the particle size.

Based on these equations seen that almost all the interaction is positive, meaning that the interaction between the components of the formulation components in

SNEDDS improving transmittance value. The interaction between the Cremophor EL with PEG 400 has a negative value. It shows that such interaction lowers transmittance value of SNEDDS. The clearer the emulsion, the smaller the particle size, and more clouded the emulsion, the bigger particle size.

pH Analysis

Nanoemulsion pH testing aims to determine the safety of nanoemulsion, especially when used on the skin. If pH value is too low it will cause irritation when used, while too high pH resulting in the scaly skin. The pH range of topical preparations is 4,5-6[9].

A coefficient value is positive, it means that the component A can increase response pH. Similarly, the coefficients B and C, but the coefficient B is greater than A and C greater than B. So this shows that the ability of the VCO component affects the pH more than Cremophor el components and PEG 400. Almost all interactions provide the coefficient positive, meaning that the interaction between components increases the pH value, except on the interaction between Cremophor EL, and PEG 400 which lowers the pH value.

Optimization of formulation

The analysis showed that the composition of the Cremophor EL is only able to form a homogeneous mixture composition if the ratio is greater than the PEG 400 and VCO. The higher the amount of surfactant in the ratio, the better the balance of interaction is achieved. Prediction optimum formulation was obtained using the design expert software version 7.0.0.

Based on analysis of the resulting superimposed contour plot of transmittance response and pH, the resulting superimposed give the yellow area which gives the optimum response. The area provides a prediction of the optimum formulation with the desirability of 0.920. The optimum composition of the formula is based on the analysis for the comparison Cremophore EL: PEG 400: VCO with a composition of 5,27: 1: 1,72.

Characterization of optimization formulation

Drug Loading

This test aims to determine the amount of ethanol that is able to extract dissolved in SNEDDS formula. The results of drug test loading of 150 mg/mL at the optimum formulation indicate that the system is not capable of dissolving extract, is characterized by the deposition on three days of observations that used the concentration of the ethanol extract of mangosteen peels is 125 mg/5 mL.

Emulsification Time

Evaluation of emulsification time using distilled water with a magnetic stirrer using a speed 120 rpm. Nanoemulsion spontaneous formation is one important parameter in the formulation SNEDDS. The measurement results in emulsification time of the optimum formulation in distilled water medium is 5.2 seconds. Co-surfactant bigger role in emulsification time. Co-surfactant will be tucked away and form the spaces between the surfactant so that its structure more bloated but has high fluidity and capable of forming nanoemulsion faster[10].

Droplet size analysis

Based on the test results, it can be seen that the average size of nanoemulsion less than 100 nm, which is 18,9 nm. This proves that the formula SNEDDS is capable of producing nanoemulsion. Theoretically nanoemulsion size greatly influenced by the ratio of surfactant to cosurfactant. PI value (polydispersity index) states nanoemulsion particle homogeneity. PI value varies from 0.0 to 1.0 and a value of 0, the more homogeneous particles[6]. PI values obtained from testing particle size distribution was 0,311.

Stability study

Stability preparations SNEDDS tested by observing the physical stability of the preparation with the active substance after storage at room temperature for three months. Observations SNEDDS stability was observed visually indicates that SNEDDS remain stable, characterized by the formation of clots or sediment on nanoemulsion.

CONCLUSION

The ethanol extract of mangosteen peels (Garcinia mangostana L.) can be formulated with SNEDDS method simplex lattice design. Based on the analysis, obtained the optimum formulation with components of Cremophor EL as the surfactant, PEG 400 as the co-surfactant, and a VCO as the oil phase with a ratio of 5.27: 1: 1.72. Evaluation SNEDDS with the optimum formulation is known drug loading value of 125 mg/5 mL, transmittance value of 74,6552%, pH value of 5,85, emulsification time of 5,2 seconds, and has a particle size of 18,9 nm, and zeta potential -6,20.

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