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Phytochemical Screening for Phenol and Optimizing the SNEDDS (Self-Nanoemulsifying Drug Delivery System) Formula Using the Simplex Lattice Design Method on *Curcuma caesia* Roxb Rhizome Extract

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Abstract
This research aims to screen the phytochemistry of phenol and optimize the formulation of *Curcuma caesia* Roxb. rhizome extract, which is difficult to dissolve in water, in the form of Self-Nanoemulsifying Drug Delivery System (SNEDDS) using the simplex lattice design method. The phenol content was identified using thin-layer chromatography,and the compound levels were determined spectrophotometrically. An initial screening was conducted to determine the oil phase, surfactant, and co-surfactant to use. The characterization of SNEDDS in the optimal formula includes transmittance, particle size, and polydispersity-index (PI). Olive oil, Tween 80,and polyethyleneglycol (PEG)-400 are the oil phase, surfactant, and co-surfactant selected due to the highest ability to dissolve *Curcuma caesia* Roxb.rhizome extract. The results of phytochemical screening of the polyphenol compound group using thin-layer chromatography produced spots with an Rf value of 0.76 and a phenol content of 61.823 mgGAE/g. The optimization results showed that the optimal formula was obtained at a composition of 1:8:1 for olive oil, Tween 80, and PEG-400. The SNEDDS of *C.caesia* Roxb.rhizome extract produced a nanoemulsion with a loading dose of 250 mg/5 g SNEDDS, a transmittance of 98.6%, a particle size of 14.1 nm, and a polydispersity index (PI) of 0.363.

Keywords: *C. caesia* Roxb, Phenol, SNEDDS

1. INTRODUCTION

The use of various plants and other natural ingredients as alternative medicines is growing increasingly for treating diseases and maintaining health, as with therapy using traditional herbal concoctions, which generally do not cause significant side effects, as is often the case with chemical treatments. The bay plant is one of the plants that has several benefits for body health. Black turmeric rhizome contains bioactive compounds such as curcuminoids, essential oils, flavonoids, polyphenols, tannins, amino acids, proteins, and high alkaloid content.

One of the contents of *Curcuma caesia* Roxb. functions as an antioxidant, able to capture free radicals, and as an anti-inflammatory and anticarcinogenic. The flavonoids, tannins, and alkaloids in the ethanol extract of *C. caesia* Roxb. are 2775.65 mg/100 grams,

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2714.75 mg/100 grams, and 1470.588235 mg/100 grams.[1] Therefore, based on these data, the rhizome of *C. caesia* Roxb. can be developed into a pharmaceutical dosage form.

Plant extracts used as one of the drug ingredients generally have poor solubility and result in low oral bioavailability. To overcome the limited solubility of *C. caesia* Roxb., one of the modifications needed is to formulate it as a Self-Nanoemulsifying Drug Delivery System (SNEDDS) dosage form. SNEDDS is an isotropic mixture of oil, surfactant, co-surfactant, and drug that spontaneously forms an O/W type nanoemulsion with a droplet size of less than 200 nm when dissolved in water with light stirring. These nano-sized droplets can increase the absorption of oil droplets due to rapid dissolution and drug release.

The optimization experimental method or design provides convenience in compiling and explaining data mathematically. The simplex lattice design method can be used to perform optimization. This method determines the optimal formula of the SNEDDS mixture by making the composition of the surfactant, cosurfactant, and oil mixture have a constant amount. The analyses to assess the quality of SNEDDS preparations are transmittance test, particle size, and polydispersity index (PI).

2. METHODS

Research Materials and Instruments :

The instruments used in this research are a 0.5-gram digital analytical balance (Precisa), a 10-mg digital analytical balance (KERN ABJ), a water bath (Grant), a micropipette (DIAB), a UV-Vis spectrophotometer (Genesys™ 10S), a vortex (Thermo), and a refrigerator (Phillips).

At the same time, the materials are *C. caesia* Roxb., Tween 80 (Duchefa, Netherlands), distilled water (Merck, Germany), 70% alcohol (Merck, Germany), methanol pro analysis (Merck, Germany), blue tip (Nesco), Ethanol (Merck, Germany), Olive oil, PEG 400 (Brataco, Indonesia), Glycerin (Brataco, Indonesia), Tween 20 (Brataco Indonesia), Tween 60 (Agung Jaya, Indonesia), Soybean oil (Agung Jaya, Indonesia), Virgin coconut oil (Agung Jaya, Indonesia).

Research Procedure :

Extracting the C.caesia Roxb. Rhizome

The cleaned *C. caesia* Roxb. was cut into 3–5 mm thick pieces and then dried in an oven at 40–50°C. The dried turmeric was then crushed using a blender and sieved using a 60-mesh sieve, in which the resulting powder was then extracted using the maceration method. The turmeric powder weighed as much as 6000 g and dissolved in 3000 ml of 96% ethanol

solvent. The turmeric powder and solvent mixture were shaken twice for five minutes and macerated for 3 x 24 hours. The solution was filtered using Whatman paper, in which the filtrate obtained was then evaporated using a rotary vacuum evaporator.

Identifying the Phytochemical Screening Compound

Phenolic Test

The 0.1-gram fraction was dissolved with 10 mL of distilled water and then filtered to obtain the filtrate. The filtrate was then reacted with three drops of 1% $FeCl_3$. The result was positive due to the appearance of blackish-blue-green color. The phenolic compound showed a black color change in the test sample but was not too concentrated after 1% $FeCl_3$ was added. A phenolic compound is declared positive if a bluish-black color changes to solid black when 1% $FeCl_3$ is added and the $FeCl_3$ can react with aromatic –OH groups.

Testing the Thin-Layer Chromatography (TLC) of the Phenol Compound

The TLC test used the Silica G60 F254 stationary phase. The TLC plate was made with 10 cm length and 3 cm width. The mobile phase used $CHCl_3$ and Methanol with a ratio of 9:1. The plate was dried and sprayed with $FeCl_3$ reagent. A positive result is indicated by the presence of dark spots (black, purple, dark blue, or dark brown).

Phytochemical Screening Quantitative Analysis: Analyzing the Total Content of Phenolic Compounds

Analysis of total phenolic compounds in ethanol extract of *C. caesia* Roxb. used the Folin-Ciocalteu method and referred to the modified research procedure as follows:

Determining the calibration curve

The gallic acid was prepared with concentration variations of 6, 12, 18, 24, and 30 ppm. Each concentration was taken at 0.5 mL and put into each test tube. 0.9 mL of distilled water and 0.5 mL of 0.25 N Folin-Ciocalteu reagent were added to the gallic acid test tube with various concentrations, then shaken and incubated for five minutes. 2.5 mL of 7% Na_2CO_3 was added to the mixture, shaken, and incubated for 20 minutes. Then, the absorbance was measured at the maximum wavelength, and a calibration curve and linear regression equation were made from the data obtained.

Determining the concentration of phenolic compounds

10 mg of *C. caesia* Roxb extract was diluted with 10 mL of ethanol solvent. The extract was taken 0.1 mL and put into a test tube. 0.9 mL of distilled water and 0.5 mL of 0.25 N Folin-Ciocalteu reagent were added, pipetted to the sample's test tube, then shaken and incubated for five minutes. 2.5 mL of 7% Na_2CO_3 was added to the tube and shaken. The mixture was incubated first for 20 minutes in a dark place at room temperature. The solution

was then put into a cuvette, and the absorbance was measured at the maximum wavelength.[11]

Testing the Extract Solubility

The screened oils were olive oil and VCO, and the surfactants used were Tween 20, 60, and 80, while the co-surfactants were Glycerin, PEG 400, and Propylene glycol. The test was done by adding 100 mg of *C. caesia* Roxb extract and 1.0 mL of each material in an Eppendorf tube; then, the mixture was vortexed for two minutes. Furthermore, the mixture was centrifuged at a speed of 3000 rpm for 20 minutes; the sediment formed was then separated from the supernatant and oven for eight hours to evaporate the material that was still mixed with the sediment, then weighed to find the insoluble extract.

Formula Optimization

The following table shows the SNEDDS system formula in 5 grams of SNEDDS, the amount of oil, surfactant, and co-surfactant used:

Table 1. Comparison ratio oil (o), surfactant (s), and co-surfactant (c)

Comparison O:S:C	Oil (gram)	Surfactant (gram)	Co-surfactant (gram)
1 : 1 : 1	1.66	1.66	1.66
1 : 2 : 1	1.25	2.50	1.25
1 : 3 : 1	1.00	3.00	1.00
1 : 4 : 1	0.83	3.34	0.83
1 : 5 : 1	0.75	3.50	0.75
1 : 6 : 1	0.60	3.80	0.60
1 : 7 : 1	0.55	3.90	0.55
1 : 8 : 1	0.50	4.00	0.50

The mixture was homogenized using a vortex for a minute, sonicated for 15 minutes, then conditioned in a water bath at a temperature of 45ºC for 15 minutes; then, each mixture was observed visually for clarity and solubility. The formula with precise results and no separation was selected. Each formula was taken 100 µl and added with distilled water up to 5 ml to see its clarity, which was measured using a UV-VIS spectrophotometer at a wavelength of 650 nm. The selected formula composition was the SNEDDS formula with a transmittance close to 100% (distilled water) and F = 1.

Testing the SNEDDS System

Measuring the Transmittance Value

100.0 µL of SNEDDS was added with 5.0 mL of distilled water in a measuring flask and homogenized by vortexing for 60 seconds, then measured using UV-VIS spectrophotometry at a maximum wavelength of 650 nm and using distilled water as a blank.

Testing the Particle Size Analyzer (PSA)

100 µL of the optimum SNEDDS formula was diluted into 5.0 mL of distilled water and mixed, then 3.0 mL was taken and put into a cuvette for analysis using the HORIBA SZ-100 instrument. The particle size data obtained as output on the computer was the average particle size and polydisperse index.

Determining the Optimum SNEEDS Formula

The optimum SNEEDS extract formula was determined based on the optimization results using the State Ease Design Expert v13 program. The independent variables were surfactant and co-surfactant compositions, and the dependent variable was clarity with transmittance measurements.

Verifying the Optimal SNEDDS Formula

The optimal formulation was verified to see the model provided by the State Ease Design Expert v13 program against the experimental results. The verification was done by comparing the experimental results and the predicted transmittance response using SPSS.

Testing the Loading Dose

This test was done by inserting the extract into 5 grams of SNEDDS. The weight of the extract inserted was 50, 100, 150, 200, 250, and 300 mg into 5 grams of optimum SNEDDS system, then vortexed for two minutes, sonicated for 15 minutes, and conditioned in a water bath at 45ºC for 10 minutes. Each dose was then stirred at 125 rpm for 30 minutes. The maximum solution of extract and fraction in the SNEDDS system was observed visually.

The Characterization of Nanoemulsion

Testing the Transmittance

The clarity/transmittance test on the emulsion was done using a UV-Vis spectrophotometer at a maximum wavelength of 650 nm. Distilled water can be used as a blank.

Testing the Particle Size

The resulting nanoemulsion formula was taken 100 µL in a 50 mL measuring flask. Then, artificial gastric fluid was added to the boundary mark/termination mark, and the droplet size and polydispersity index (PI) were calculated.

3. RESULTS AND DISCUSSION

Testing the Phytochemical Screening

The phytochemical screening process can reveal the group of compounds contained in the ethanol extract of *C. caesia* Roxb. Table 2 shows the results of phytochemical screening on the ethanol extract of black turmeric, which were positive for phenolic compounds.

Table 2. Result of testing the phytochemical screening

Parameter	Reaction	Indicator	Result
Fenol	Extract + FeCI3	Green, red, purple, blue or deep black	+

Description:
Positive (+) : contains secondary metabolite compounds (phenol)

Qualitative Analysis with Thin-Layer Chromatography (TLC)

The TLC test was carried out to confirm the results obtained from phytochemical screening further. It used several combinations of eluents to separate phenolic compounds, including chloroform : methanol = 9:1. The stationary phase used was a silica gel G60F254 plate. The separation results in the form of spots were calculated to obtain the Rf value, and the compounds were identified using the TLC color results along with the Rf value.

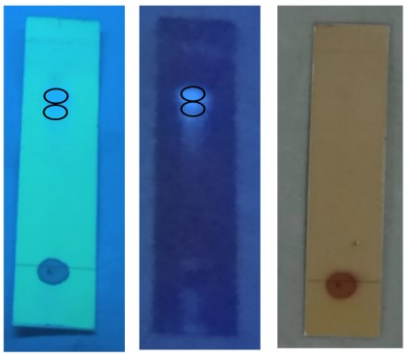

Figure 1. Results of identifying phenolic compounds: (a) Observation of UV light 254, (b) observation of UV light 365 nm, (c) visual observation

Table 3. Thin layer chromatography screening of phenolic compounds

Sample	Spot No	Color spot + Rf		
		UV 254 nm	UV 366 nm	Visual FeCI3 2%
C.caesia extract	1	blue 0,67	purple 0,67	-
	2	blue 0,76	purple 0,76	-
Gallic acid marker		blue 0,76		

Table 3 shows that black turmeric extract contains phenolic compounds. The Rf value of 0.76 in the separation of phenolics is like the Rf value of gallic acid of 0.76, which indicates the presence of phenolic compounds.

Quantitative Analysis with UV-Vis Spectrophotometer

Determining the Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu method, whose principle is the oxidation and reduction reactions between phenolic compounds and Folin-Ciocalteu reagents. The reaction between phenolic compounds and Folin-Ciocalteu reagents can only occur in a base atmosphere. Therefore, 7% Na_2CO_3 is needed to make the solution base.

The standard for determining the total phenolic content is gallic acid, a simple phenol compound derived from hydrobenzoate. Gallic acid has pure and stable properties, so it can be used as a comparative solution. The standard solution was made with 6, 12, 18, 24, and 30 ppm series to produce a linear regression equation from the gallic acid calibration curve. The absorbance of the standard solution concentration series after incubation for an operating time of 90 minutes and at a maximum wavelength of 715 nm.

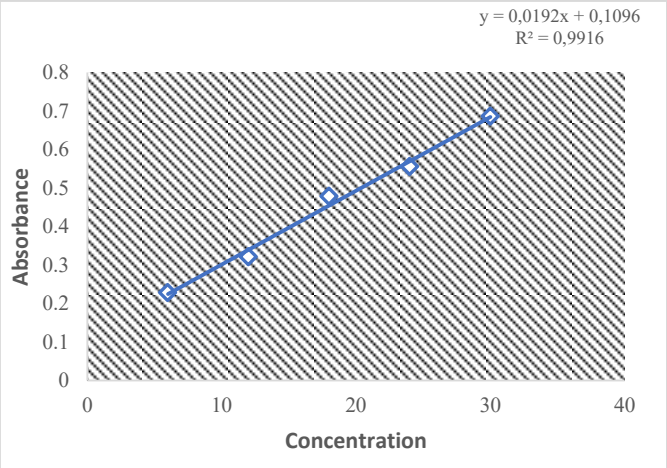

Figure 2. Gallic acid calibration curve

Based on the measurements, a linear equation of gallic acid was obtained: y = 0.0192x + 0.1096 with an R2 value of 0.9916. This linear regression equation compares the total phenolic content in the extract of *C. caesia* Roxb. The results of determining the phenolic content of the ethyl acetate fraction and the methanol fraction of corn husk can be seen in Table 4, which shows the results of 61.823 mgGAE/g.

Table 4. Result of the total phenolic content of thr extract of *C. caesia* Roxb.

Absorbance	Phenolic Content (mgGAE/g)	Average Phenolic Content (mgGAE/g)
0,347	61,823	61,823
0,346	61,563	
0,348	62,083	

Testing the Black Turmeric Extract Solubility

Oil, surfactant, and cosurfactant solubility tests were conducted to determine the appropriate oil, surfactant, and cosurfactant to be formulated with ethanol extract of *C. caesia* Roxb rhizome. The extract solubility test was conducted by dissolving the ethanol extract of black turmeric in the tested oil, surfactant, and cosurfactant, where the selected oil, surfactant, and cosurfactant were those that could dissolve the ethanol extract of *C. caesia* Roxb. the most. The oils used for the solubility test were olive oil, virgin coconut oil, and soybean oil.

The surfactants used for screening were Tween 20, tween 60, and Tween 80, while the cosurfactants were PEG 400, glycerin, and Propylene glycol. Based on the solubility test results in Table 2, olive oil was selected, with Tween 80 as the surfactant and PEG 400 as the cosurfactant. The best results were selected based on their most significant solubility. The oil, Surfactant, and Cosurfactant selected are olive oil, Tween 80, and PEG 400. The chosen oil, surfactant, and cosurfactant were optimized using several comparisons to obtain the best ratio.

Table 5. Result of testing the *C. caesia* Roxb. Extract solubility

C. caesia Roxb. extract (mg/ml)		Solubility (mg/ml)
100	**Oil**	
	Soybean Oil	76,19
	Olive Oil*	86,95
	VCO	85,71
	Surfactant	
	Tween 20	90,91
	Tween 60	76,19
	Tween 80*	95,23
	Co-Surfactant	
	PEG 400*	95,45
	Glycerin	84,00
	Propylene Glykol (PG)	91,30

Table 6. Composition ratio of oil, surfactant and co-surfactant

O:S:C	Oil (gram)	Surfactant (gram)	Co-surfacrant (gram)	Homogeneity	Stability	Transmittance (%)	Description
1 : 1 : 1	**1.66**	1.66	1.66	Not homogeneous	0,66	1,60	Not clear
1 : 2 : 1	**1.25**	2.50	1.25	Not homogeneous	0,71	5,76	Not clear
1 : 3 : 1	**1.00**	3.00	1.00	Not homogeneous	0,77	50,40	Not clear
1 : 4 : 1	**0.83**	3.34	0.83	Not homogeneous	0,86	51,30	Not clear
1 : 5 : 1	**0.75**	3.50	0.75	Not homogeneous	0,89	53,50	Not clear
1 : 6 : 1	**0.60**	3.80	0.60	Not homogeneous	0,90	54,00	Not clear
1 : 7 : 1	**0.55**	3.90	0.55	Homogeneous	0,92	71,20	Quite clear
1 : 8 : 1	**0.50**	4.00	0.50	Homogeneous	1	98,00	Clear

SNEDDS System : 5 g

Oil (O) : Olive oil

Surfactant (S) : Tween 80

Co Surfactant (C) : PEG 400

The composition of the oil phase, surfactant, cosurfactant, and extract in the SNEDDS formulation will directly form a homogeneous and clear liquid when mixed with the water phase. The compatibility between oil, surfactant, and cosurfactant is the basis for determining the composition of SNEDDS to form nanoemulsions. The oil phase must first be coated by surfactants and cosurfactants.

Table 6 shows that the 1 : 8 : 1 ratio gives precise results with a transmittance of 98.00%. The formula composition with a 1 : 8 : 1 ratio is the best. This is based on the composition of surfactants that can form a visually apparent nanoemulsion and a transmittance value approaching 100%. A good nanoemulsion has an evident visual appearance with a more than 90% transmittance value. Therefore, the formula chosen is a formula with a ratio of surfactants and cosurfactants that has provided precise visualization results and has a transmittance value of more than 90.

The amount of cosurfactant in this research was PEG 400, less than Tween 80, which acts as a surfactant. Excessive use of cosurfactants will cause the system to become less stable. This is due to the high intrinsic solubility found in water, which triggers an increase in particle size because of the expansion of the interface film layer.

The SNEDDS experimental formula design from the State Ease dx-9 Trial Program

Determining the olive oil component as the oil phase, Tween 80 as a surfactant, and PEG 400 as a co-surfactant is the basis for optimizing the SNEDDS formula using the State Ease Design Expert V13 program. The independent variable components used are the composition of Tween 80 and PEG 400, made into eight trial runs by the State Ease dx 9 trial programs as in the table.

The first step in testing the characteristics of SNEDDS is testing the clarity, which is to determine whether the preparation produced by the SNEDDS formula has formed nanometer-sized droplets that are visually clear and can be observed with a UV Vis spectrophotometer.

Table 7. The stability test results and transmittance values of olive oil, tween 80, and peg 400 compositions

RUN	Compesition Code		Composition (%)		Amount (gram)		Oil (gram)	F Value	HLB	Transmittance t(%)
	S	K	S	K	S	K				
1	0	1	88,89	11,11	4	0,5	0,5	1	14,62	92,9
2	0,5	0,5	93,05	6,95	4,19	0,31	0,5	1	14,76	95,3
3	0	1	88,89	11,11	4	0,5	0,5	1	14,62	92,7
4	0,5	0,5	93,05	6,95	4,19	0,31	0,5	1	14,76	93,8
5	0,75	0,25	95,14	4,86	4,28	0,22	0,5	1	14,83	97,5
6	1	0	97,22	2,78	4,38	0,12	0,5	1	14,91	99,2
7	0,25	0,75	90,97	9,03	4,09	0,41	0,5	1	14,69	92,5
8	1	0	97,22	2,78	4,38	0,12	0,5	1	14,91	99,7

Based on the table, it is discovered that the emulsion formed has a transmittance value above 90%. The emulsion with clarity and a transmittance value that is increasingly close to the transmittance value of distilled water (100%) indicates that the droplets formed are increasingly smaller in nanometer size. The nanoemulsion formed is greatly influenced by the system's HLB value; a good nanoemulsion has a system HLB value between 12-20. The increase in HLB of the SNEDDS preparation system will quickly form an M/A type emulsion when it meets the media, which will reduce the average size of the emulsion droplets produced. The high transmittance value of the SNEDDS formula of *C. caesia* Roxb. extract indicates that the emulsion droplets formed are tiny (nanometers).

The requirement for analysis using the ANOVA statistical program is that the resulting data be normally distributed. Figure 3 is a typical residual clarity response graph plot showing that the resulting data is normally distributed. The observation data is evenly distributed around the diagonal line and follows the direction of the diagonal line, so it is said that the data is normally distributed.

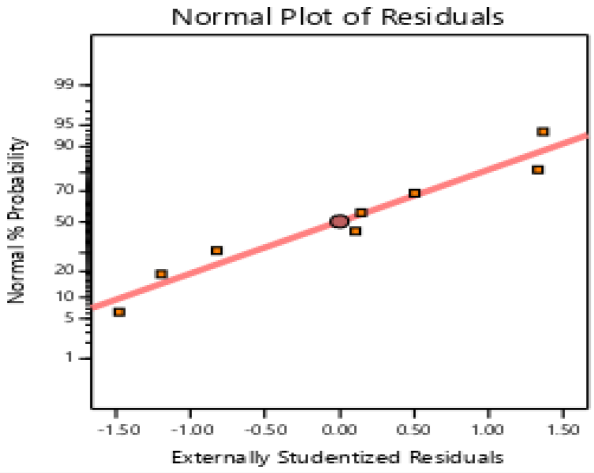

Figure 3. Standard plot of residuals results of the snedds formula transmittance response test

The results of the ANOVA statistical analysis showed that the obtained model was significant with a 95% confidence level, a calculated F-value of 58.83, and a p-value of less

than 0.05 (0.0003), which indicates that the quadratic model is the suitable model in explaining the results of the transmittance value test. For the lack of fit analysis, the calculated F-value was 1.48, and the p-value was greater than 0.05 (0.3576), indicating no significant difference between the observation data and the data predicted by the model. An analysis was carried out on the results of the clarity response test using the State Ease Design Expert V13 program to determine the effect of components on clarity. From the analysis, a quadratic equation is obtained as shown in the equation:

$$Y = 99{,}6405\ A + 92{,}6183\ B - 6{,}21176\ AB \quad \ldots\ldots\ (1)$$

Description :

Y = Transmittance (%T)

A = Tween 80 composition

B = PEG 400 composition

The quadratic models show that the Tween 80 and PEG 400 components with large coefficient values positively influence the transmittance response, meaning each component can increase the clarity response. In addition to the influence of the two elements, the transmittance response is also influenced by the interaction of Tween 80 and PEG 400. The negative sign in equation *(1)* indicates that the interaction of Tween 80 and PEG 400 negatively influences the response; that is, it can reduce the resulting transmittance value. Furthermore, it can be estimated that the influence of each component is more significant than the interaction of the two elements on the clarity response. The negative sign describes the negative influence on the reaction, which can reduce the transmittance value.

Figure 4 shows the relationship between the Tween 80 surfactant and the PEG 400 co-surfactant on the clarity response. The curve of the two-component mix shows that the system with a more significant amount of surfactant produces clearer SNEDDS compared to the system with a more substantial amount of co-surfactant.

Optimum Formula

An optimum SNEDDS formula is determined using the determining response: clarity. The optimum SNEDDS formula was determined using the State Ease Design Expert V13 program by specifying parameter values, such as the limits of the response components: maximum, minimum, in range, and none. The Tween 80 and PEG 400 components were selected as in-range limits to obtain the component composition within the previous formula run range. In the clarity response, the maximum limit is selected so that the chosen optimum formula has a high transmittance value that can form a clear nanoemulsion.

The State Ease Design Expert V13 program provides a solution for the selected

formula with a desirability or optimization target value. The desirability value indicates a tendency for the response results to be achieved following the desired optimization target. The desirability value ranges from 0 to 1, where the desirability value closing to 1 indicates that the formula is increasingly under the desired optimum formula based on the specified variables.

Table 8 shows that the optimum SNEDDS formula is selected with a composition of 97.220% Tween 80 and 2.780% PEG 400, with a desirability value approaching one, namely 0.992. The selection of the optimum composition is based on the clarity of the response results. The State Ease Design Expert V13 program provides a predicted response value of 99.641% for the clarity response on selected optimum formula. The result obtained from testing the two responses were compared with the expected result using a one sample t-test in the SPSS v21 program.

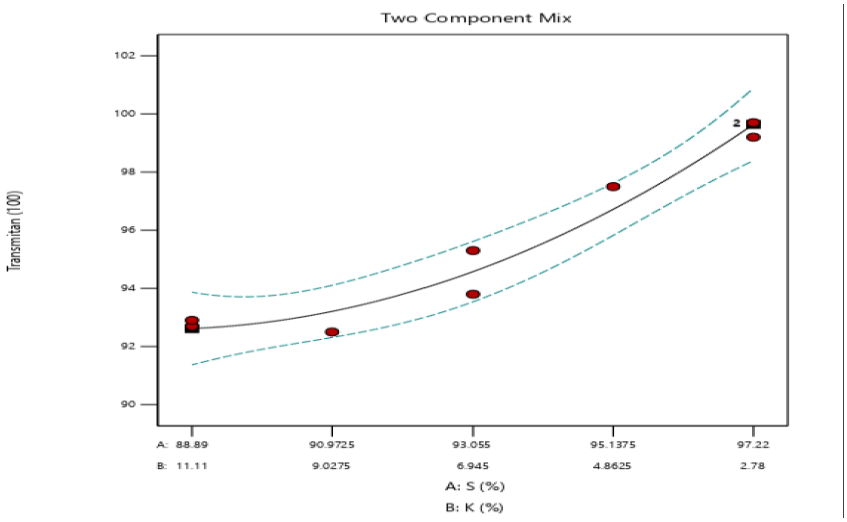

Figure 4. The curve of the two-component mix of the transmittance response test results of the *c.caesia* ethanol extract snedds formula

Table 8. The optimum SNEDDS formula

Composition of Simplex Lattice Design (SLD)		Composition (%)		Transmittance (%)	*Desirability*	Description
S	C	S	C			
4,38	0,12	97,220	2,780	99,641	0,992	***Selected***

*) S is Tween 80 , C is PEG 400

Verifying the Optimum SNEDDS Formula

Testing the clarity of the optimum SNEDDS formula is necessary to prove that the resulting emulsion is visually clear and can be observed spectroscopically. As seen in Table 9, the optimum SNEDDS formula has a transmittance value of 99.767%, which indicates that the resulting emulsion has good clarity.

Table 9. The results of verifying the optimum snedds formula

Composition (%)		Transmittance (%)			Average ± SD (%)	Prediction of SLD	Sig. (2-tailed)
S	C	1	2	3			
97,22	2,78	99,800	99,800	99,700	99,767	99,641	1,00

The results of the transmittance value of the optimum SNEDDS formula were analyzed using a one-sample t-test in the SPSS v21 program. The Sig. (p-value) value was obtained greater than 0.05, namely 1.00, which indicates that H_0 is accepted. These results suggest that the clarity test on the optimum SNEDDS formula does not significantly differ from the State Ease Design Expert V13 program's predictions. Thus, it can be said that the quadratic model is the right one to use in optimizing the SNEDDS formula for clarity response.

Testing the Loading Dose

Drug entry into the system is critical because the drug can affect the emulsification process, which triggers changes in the optimal ratio of oil, surfactant, and co-surfactant. Extract loading optimization is needed to determine the maximum amount of extract that can be carried or dissolved in an SNEDDS system to have an optimal therapeutic effect and form a stable nanoemulsion.

Table 10 shows that a dose of 250.0 mg can still be dissolved in the optimum SNEDDS system, evidenced by the absence of sediment formation after a day of visual observation

Table 10. The results of testing the extract loading of the snedds optimum formula for the extract

Concentration of *C. caesia* Roxb.extract (mg/5,0 g system SNEDDS)	Result
50	Dissolved
100	Dissolved
150	Dissolved
200	Dissolved
250	Dissolved
300	There is sediment

Nanoemulsion Characterization

Characterizing the particle size in the optimum formula of SNEDDS extract *C. caesia* Roxb. is necessary to ensure that the emulsion formed is a nanoemulsion and to determine the uniformity of the size of the resulting nanoemulsion droplets. Droplets are considered nanometer-sized if their size is less than 100 nm.[20]

Table 11. The results of testing the size and distribution of nanoemulsion droplets of the snedds optimum formula snedds for the *c. Caesia* roxb. Extract

Dose (mg/5,0 g System SNEDD)	% Transmittance	Nanoemulsion droplet size (nm)	*Polysdispersity Index*
250	98,6	14,1	0,363

The percentage transmittance test determines the clarity of the nanoemulsion formed, where the ideal transmittance value in nanoemulsion ranges from 90-100%. This transmittance range is the optimal nanoemulsion visual, shown clearly and transparently. The test results show that the black turmeric extract nanoemulsion has a transmittance value of 98.6%. This indicates the formation of a nanoemulsion that can produce small droplet sizes. The higher the percent transmittance value indicates, the finer the size of the nanoemulsion droplets formed. The high percent transmittance results of the nanoemulsion indicate that the nanoemulsion formed appears clear because the tiny droplet size can pass through the light beam, thus indicating a high transmittance measurement.

Table 11 shows that the emulsion produced by the optimum formula at a dose of 250.0 mg/5.0 grams of the SNEDDS system has a transmittance value of 98.6%, a droplet diameter size below 100 nm, which is 14.1 nm, with a polydispersity index (PI) of 0.363. The results indicate that the resulting nanoemulsion has a droplet size following the characteristics of the nanoemulsion and a polydispersity index (PI) close to zero. The low value of the resulting polydispersity index (closer to zero) indicates that the uniformity of the globule size in the preparation is getting higher.

4. CONCLUSION

The phytochemical screening of polyphenolic compounds in *C. caesia* Roxb. extract using thin-layer chromatography produces a spot with an Rf value of 0.76 and a phenol content of 61.823 mgGAE/g. The optimum SNEDDS of *C. caesia* Roxb. extract in a ratio of 2.780% olive oil, 97.220%, Tween 80, and 2.780% PEG 400 can produce a homogeneous mixture with a transmittance value of 99.767%T, and can carry 250 mg of black turmeric extract per 5 grams of SNEDDS system. The optimal SNEDDS has a nanoemulsion size characteristic of 14.1 nm with a polydispersity index (PI) of 0.363.

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