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Evaluation of Self Nanoemulsifying Drug Delivery System from Qusthul Hindi (*Saussurea lappa*) Extract: In vitro Release and Absorption Assessment

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This study aimed to determine the release and absorption flux values of qusthul Hindi (Saussurea lappa) self nanoemulsifying drug delivery system using the template formula for the optimal D-optimal design approach. The method is emulsification. The in vitro release test used the dialysis bag method. The absorption test was carried out through the intestinal membrane using the Crane and Wilson tube method. The levels released and absorbed were measured using a UV-Vis Spectrophotometer. The results showed that in vitro release and absorption values in SNEDDS were higher than the qusthul Hindi (Saussurea lappa) extract solution in both artificial intestinal fluid media at pH 6.8 ± 0.05 and artificial gastric fluid at pH 1.2 ± 0.05. The analysis found a significant difference in flux for both release (p=0.02) and absorption (p=0.01). The SNEDDS formula provided in vitro release flux results that were 4.20 times higher in AGF media and 5.62 times higher in AIF media compared to the extract solution. In vitroabsorption results also indicated a 4.02 times increase in AGF media and 3.32 times higher in AIF compared to the extract solution. The selected formulas using miglyol 812, Tween 80, and Polyethylenflycol 400 components at concentrations of 2.13%, 5.81%, and 2.06% can be used for SNEDDS formulations of qusthul Hindi (Saussurea lappa). These formulations showed superior in vitro release and absorption results compared to the extract solution in both AIF media at pH 6.8 ± 0.05 and AGF media at pH 1.2 ± 0.05.

Keywords: Absorption test; Qusthul Hindi; Release test; Saussurea lappa; Self-nanoemulsifying.

Pharmaceutical formulation technology and drug delivery systems are essential in discovering new pharmaceutical therapies[1] . Developing a drug delivery system generally takes a long time because many variables must be optimized to obtain a formula that meets predetermined specifications. A drug delivery system can be used to develop chemotherapy drugs or cancer treatments that kill abnormal cells in the body, so it is necessary to develop technology to reduce side effects [2] .

Applying drug delivery systems to natural products has several advantages, including increasing solubility, increasing bioavailability, reducing toxicity, increasing pharmacological activity, increasing stability, and preventing

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physical or chemical degradation[3]. Preparations based on drug delivery systems can be an alternative to pharmaceutical preparations that contain natural ingredients.

Based on data on the solubility and pharmacological activity of Qustul Hindi extract, it is necessary to develop formulation technology to increase its solubility, stability, and bioavailability to maximize the therapeutic effect by using the extract as a medicinal compound. One technology widely used in formulation is nanoparticle technology, which has now become a model for developing drug delivery systems[4]. Research on the ability of SNEDDS to increase the bioavailability of drugs with low solubility in systemic circulation has been widely proven compared to conventional preparations such as tablets.

Using nanotechnology, bioactive components from herbal ingredients can be isolated and encapsulated into nanoparticles to increase drug availability and facilitate drug delivery to the target location. Therefore, in this research, researchers will develop a standardized formulation and downstream nanotechnology preparation of qusthul Hindi as a chemopreventive agent to minimize the side effects of chemotherapy [4].

Qusthul Hindi, indigenous to the Himalayan region, has gained attention due to its various metabolites, including sesquiterpenes, flavonoids, phytosterols, lignans, and terpenes, which have pharmacological effects as chemotherapy agents. To further validate these components and subsystems, metabolite profiling will be conducted to determine significant compounds in the extract responsible for its pharmacological activity, specifically as chemopreventive. Therefore, this study aimed to determine the release and absorption flux values of qusthul Hindi SNEDDS using the template formula for the optimal D-optimal design approach.

MATERIAL AND METHODS

Materials

The main ingredient used in this study was the qusthul Hindi extract obtained from Saudi Arabia as costus root powder. The simplicity was identified in the pharmaceutical biology laboratory, Faculty of Medicine and Health Sciences, UIN Maulana Malik Ibrahim Malang, using macroscopic and microscopic approaches to ensure the accuracy of the name. The specimen was labeled with the number 075/212/102 20-A/2022.

Ingredient formulation comprised Miglyol 812 (Sigma Aldrich, USA), Tween 80 and PEG 400 (Merck, Germany), Ethanol 96% (Smartlab, Indonesia), HCl, NaOH, KH_2PO_4, HCl, NaCl, $MgCl_2$, $CaCl_2$, $NaHCO_3$, KCl, $NaHPO_4$ pro analysis (Merck, Germany).

Research Design

This study used an experimental laboratory design focused on SNEDDS formulations using different active ingredients. The optimal formula template from a previous investigation was achieved with the optimal D-design approach using Design-Expert®12 software[5] . This study used further formula application with a different active ingredient, qusthul Hindi. The effectiveness examinations carried out were release and absorption tests. The release test used the dialysis bag method, while the absorption test used the reverse gut technique.

Method

Preparation of Qusthul Hindi (*Saussurea lappa*) Extract

Extraction was carried out using the maceration method with the help of ultrasound-assisted extraction. Qustul Hindi was extracted thrice using 500 mL of 96% ethanol at room temperature at 10-minute intervals. Approximately 25 grams of sample was dissolved in 500 mL of 96% ethanol at a ratio of 1:20. After obtaining the liquid extract, it was concentrated using a rotary evaporator (Heidolph, Germany). After obtaining a thick extract, an ethanol-free examination is carried out to ensure no solvent influence in the extract. This examination is carried out by adding H_2SO_4 (p) to the extract, then adding CH3COOH, then heating. The test result is negative if there is no characteristic ester odor.

Formulation of SNEDDS Qusthul Hindi (*Saussurea lappa*) Extract

The SNEDDS formula was prepared using the D-optimal design approach based on a previous template[6]. The components included 50 mg of qusthul Hindi extract, 2.13%, Miglyol 812 (oil component), 5.81% Tween 80 (surfactant), and 2.06% PEG 400 (co-surfactant)[7–9].

Preparation for Making Release and Absorption Test Media

The media used in this study were artificial

gastric fluid (AGF) at pH 1.2 ± 0.05 and artificial intestinal fluid (AIF) at pH 6.8 ± 0.05. As illustrated in Table 1, the media were prepared by dissolving each ingredient with distilled water, and then the degree of acidity of the solution was measured with a pH meter[10].

Preparation of Release and Absorption Test Membrane

The release membrane used in the test was a 12,000 g/mole dialysis bag with a 0.45 ìg filter. The preparation method is to cut the membrane 5 cm long and then soaked in distilled water for (±12 hours). Immediately before use, the membrane is drained until no water sticks to it. A dialysis bag ready to be filled with 1 gram of sample is then tied at the bottom and top ends using thread. Before using the dialysis bag, ensure that the dialysis bag filled with the sample does not leak.

The absorption test membrane was used in rat intestines. Experimental animals were fasted for 20 - 24 hours but were given boiled water. The mice were euthanized using ether under anesthesia for 20 minutes, then their stomachs were opened along the linea mediana, and the intestines were removed. The intestine was removed 15 cm below the pylorus, and 20 cm below it was cut for experiments. The intestine is divided into two equal parts and then cleaned. The anal section was used as a control. The anal end of the piece of the intestine is tied with thread; then, using a glass rod with a diameter of 2 mm, the intestine is inverted so that the mucosa is located outside.

The Release Test of Qusthul Hindi (*Saussurea lappa*) Extract

The release test used a type 2 paddle type dissolution equipment[8], and the media used was 500 mL with a temperature of 37oC ± 0.5oC. The dialysis bag containing the sample was inserted into a chamber containing 500 mL of artificial gastric fluid (AGF) at pH 1.2 ± 0.05, and the device was run. The release media used were AGF, at pH 1.2 ± 0.05, and artificial intestinal fluid (AIF), at pH 6.8 ± 0.05. Stirring was carried out at a speed of 100 rpm to maintain the homogeneity of the release medium. The distance of the paddle base from the bottom of the chamber was 2.5 ± 0.2 cm, and the release test was carried out for 4 hours. Sampling was conducted at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, and 240 minutes with the number of samples per collection being 3 ml. The release media was added at each sampling with a 3 mL AGF solution volume. The samples taken were then filtered, and the absorbance of the filtrate was measured using a spectrophotometer (Shimadzu UV-1800) at the maximum wavelength. In addition, the release test was carried out in three replications, while the absorbance results were used to calculate the cumulative amount at a specific time interval. The levels were calculated using the standard curve equation, and a release profile was created by entering the levels released at a particular time. The steps were repeated in AIF pH 6.8 ± 0.05[9] for the release test.

In vitro study for Absorption Testing of SNEDDS Qusthul Hindi (*Saussurea lappa*)

Absorption testing was carried out using Crane and Wilson tubes. The cannula is inserted into the oral end of the unattached intestine. The intestine was measured with an effective length of 7 cm, which was previously filled with 1.4 mL serosal fluid consisting of 0.9% w/v NaCl solution. The intestinal bag filled with serosal fluid is put into a tube filled with 75 mL of artificial gastric fluid (AGF) pH 1.2 ± 0.05 (which contains SNEDDS extract of qustul Hindi at a temperature of 37°C. Intestinal control bags were carried out the same way but using SNEDDS without Qustul Hindi extract. During the experiment, all parts of the intestine were submerged in mucosal fluid and continuously supplied with oxygen gas at approximately 100 bubbles per minute. The drug level in the serosal fluid is determined at a particular time. For this determination, 1 mL of serosal fluid is taken through a cannula and filled with 1 mL of 0.9% w/v NaCl solution. The absorption test is carried out for 4 hours. Samples are taken in minutes 10, 20, 30, 40, 50, 60,70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, and 240. The absorption test was carried out three times. The absorbance results were used to calculate the cumulative amount at a specific time interval. The levels are calculated using the standard curve equation, and a release profile is created by entering the levels released at a particular time. The same steps were repeated for absorption testing using artificial intestinal fluid (AIF) medium pH 6.8 ± 0.05 [9].

Data Analysis

Determination of the cumulative amount

released/absorbed from SNEDDS per unit membrane area at any time was calculated from the levels obtained at each time (ìg/mL) correlated using the Wurster formula multiplied by the amount of media and divided by the membrane surface area. Subsequently, a curve was created between the cumulative amount released/absorbed (ìg/cm2) versus time. A regression equation was obtained based on the resulting curve: y = bx + a. According to Fick's law of diffusion, the slope of the regression equation is the speed of release and absorption.

The Wurster formula equation is as follows:

Cn = C'n + $^{VS}\Sigma^{N-1}$ $C_{SV}M^{S-1}$

Cn = Actual levels after correction (ppm)

C'n = Readable levels (calculation results from the sample absorption values read on the spectrophotometer in ppm

Cs = Readability from previous samples

Vs = Sample volume taken

Vm = Media volume

RESULTS AND DISCUSSION

The Release Test of SNEDDS Qusthul Hindi (*Saussurea lappa*)

Results of determining the average cumulative amount of qustul Hindi extract released in SNEDDS qustul Hindi extract and qustul Hindi extract solution on AGF media 1.2 ± 0.05 and AIF 6.8 ± 0.2 can be seen in Table 2. The results of the cumulative amount released are then used to create a curve relating the cumulative amount of drug released to time (minutes). The release test was replicated three times for SNEDDS of qustul Hindi extract and qustul Hindi extract solution, which was carried out for 4 hours. The initial concentration of the extract used in the test was 5000.045 ìg and 5004.133 ìg for the SNEDDS extract of qustul Hindi. The released qustul Hindi extract was calculated to obtain a cumulative amount over 4 hours. Figure 1 shows the release profile of qustul Hindi extract and qustul Hindi extract solution on AGF 1.2 ± 0.05 and AIF 6.8 ± 0.2 media. Based on the release profile and continued calculation of the release flux, SNEDDS of qustul Hindi extract provides a higher release of 3.82 times than qustul Hindi extract solution on AGF 1.2 ± 0.05 and AIF media. 6.8 ± 0.2.

The release movement that occurred in the test was observed for 240 minutes. Based on curve one, there was an increase in the cumulative amount released on a flat curve. This indicates a difference in the cumulative amount of drug content released in the AGF medium pH 1.2 ± 0.05 and AIF pH 6.8 ± 0.05. It can be concluded that the results of the SNEDDS extract of qustul Hindi on AIF media provide a higher release than on AGF media. This is possible because the number of hydronium ions in the pH 1.2 ± 0.05 medium could not significantly release the qustul Hindi extract in SNEDDS. The increase in the cumulative amount of drug

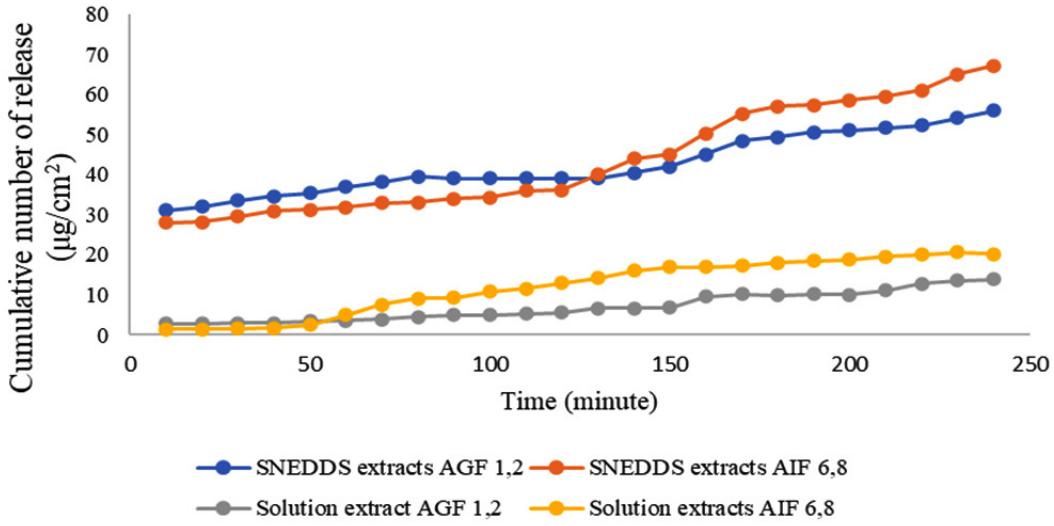

Fig. 1. Graph of the cumulative amount of qusthul Hindi extract (µg/cm²) released

content released in media pH 6.8 ± 0.05 is due to the solubility of SNEDDS extract of qustul Hindi used in this study, namely 7.04 - 7.33, causing a tendency for more neutral solubility at pH 6, 8 ± 0.05. The release profile in AGF media pH 1.2 ± 0.05 and AIF pH 6.8 ± 0.05 occurred following the first-order reaction. The conclusion that can be drawn at the initial contact of SNEDDS with the media is that the release is influenced by the ability of the media solution to dissolve SNEDDS. The better the ability, the greater the drug that can be released. From the release data in Table 1, a linear regression equation was created between the cumulative amount (ìg/cm^2) and the time (minutes) released. The discharge flux value is the slope of the linear regression equation. The SNEDDS release flux values of qustul Hindi extract. The qustul Hindi extract solution can be seen in Table 3.

Based on the two-way ANOVA statistical analysis of the release test flux (p=0.02), there was a significant difference between the SNEDDS and Qusthul Hindi extract solutions in AIF and AGF media.

In Vitro Study for Absorption Testing of SNEDDS Qusthul Hindi (*Saussurea lappa*)

Table 4 shows the average amount absorbed in SNEDDS and Qusthul Hindi extract solution on AGF and AIF media. The results were then used to create a curve relating the cumulative amount of drug absorbed to time (minutes).

The absorption test was replicated three times for SNEDDS of qustul Hindi extract and qustul Hindi solution, which was carried out for 4 hours. The initial concentration of the extract used in the test was 67,240 ìg and 66,700 ìg for the SNEDDS extract of qustul Hindi. The absorbed qustul Hindi extract was calculated to obtain the cumulative amount for 4 hours. Figure 2 shows the absorption profile of qustul Hindi extract and qustul Hindi extract solution on AGF 1.2 ± 0.05 and AIF

Table 2. The cumulative amount of qustul Hindi extract (ìg/cm^2) released (p-value 0.05)

Time (minute)	Cumulative amount of SNEDDS extracts qusthul Hindi shedding (µg/cm^2)*		Cumulative amount of extract solution qusthul Hindi shedding (µg/cm^2)*	
	AGF 1.2 ± 0.05	AIF 6.8 ± 0.2	AGF 1.2 ± 0.05	AIF 6.8 ± 0.2
10	31.081 ± 1.008	27.991 ± 0.054	2.874 ± 0,289	1.511 ± 0.166
20	32.049 ± 1.949	28.191 ± 0.150	2.898 ± 0,381	1.469 ± 0.078
30	33.503 ± 0.607	29.491 ± 0.041	2.930 ± 0,302	1.667 ± 0.156
40	34.646 ± 0.970	30.911 ± 0.015	3.045 ± 0,545	1.817 ± 0.076
50	35.303 ± 1.284	31.291 ± 0.025	3.408 ± 0,569	2.544 ± 0.179
60	36.959 ± 0.140	31.901 ± 0.015	3.611 ± 0,733	4.955 ± 0.722
70	38.138 ± 0.727	32.910 ± 0.015	3.897 ± 0,423	7.568 ± 0.117
80	39.492 ± 1.871	33.091 ± 0.021	4.522 ± 1,262	9.087 ± 0.407
90	39.110 ± 1.259	33.941 ± 0.015	4.900 ± 1,170	9.267 ± 0.511
100	31.081 ± 1.008	34.291 ± 0.251	5.018 ± 1,030	10.840 ± 0.239
110	32.049 ± 1.949	35.911 ± 0.235	5.339 ± 1,498	11.650 ± 1.117
120	33.503 ± 0.607	36.111 ± 0.148	5.557 ± 1,652	12.942 ± 0.686
130	34.646 ± 0.970	47.141 ± 0.214	6.610 ± 0,195	14.240 ± 0.663
140	38.691 ± 5.120	48.951 ± 0.154	6.592 ± 0,121	16.071 ± 0.352
150	45.438 ± 1.064	49.991 ± 0.457	6.741 ± 0,737	16.901 ± 0.385
160	47.513 ± 3.151	50.191 ± 0.450	9.558 ± 2,443	16.919 ± 1.077
170	48.439 ± 3.638	55.221 ± 0.542	10.235 ± 0,912	17.322 ± 0.755
180	49.287 ± 4.094	56.991 ± 0.012	9.918 ± 0,792	18.011 ± 0.306
190	50.639 ± 1.581	57.351 ± 0.002	10.266 ± 0,768	18.512 ± 1.212
200	50.999 ± 1.314	58.491 ± 0.050	10.110 ± 1,887	18.728 ± 0.582
210	51.607 ± 1.331	59.491 ± 0.208	11.121 ± 0,092	19.519 ± 0.859
220	52.297 ± 1.483	60.991 ± 0.158	12.811 ± 4,084	20.026 ± 0.687
230	54.157 ± 2.057	65.021 ± 0.152	13.516 ± 0,629	20.630 ± 1.412
240	55.950 ± 2.196	67.191 ± 0.068	13.897 ± 1,715	20.228 ± 0.729

*Data are expressed as mean±SD, n=3

6.8 ± 0.2 media. Based on the absorption profile and followed by the absorption flux calculation, Table 5 shows that SNEDDS of qustul Hindi extract provides 4.66 times higher absorption than qustul Hindi extract solution on AGF 1.2 ± 0 media. 05 and AIF 6.8 ± 0.2.

Results of determining the average amount of qustul Hindi extract absorbed in SNEDDS qustul Hindi extract and extract solution. Based on the absorption profile and continued absorption flux calculations, it shows that SNEDDS of qustul Hindi extract provides 4.66 times higher absorption compared to qustul Hindi extract solution in AGF medium pH 1.2 ± 0.05. The absorption data in Table 4 created a linear regression equation between the cumulative amount (ig/cm2) and the time (minutes) absorbed. The absorption flux value is the slope of the linear regression equation. The SNEDDS absorption flux value can be seen in Table 5. It was obtained based on the two-way ANOVA statistical analysis of the absorption test ($p > 0.05$). This shows a significant difference between the SNEDDS absorption flux of qustul Hindi extract and qustul Hindi extract solution in AIF medium pH 6.8 and AGF 1.2. From the results of determining the absorption flux value, the reverse rat intestinal permeability value was calculated for the absorbed qustul Hindi extract by dividing the absorption flux by the level of qustul Hindi extract used. The permeability value of the rat intestine to the absorbed qustul Hindi extract can be seen in Table 6. It was obtained based on the two-way ANOVA statistical analysis of permeability calculations ($p = 0.02$). This shows that there is a significant

Table 3. The release flux (µg/cm²/minute) of SNEDDS and qusthul Hindi extract solution

Media	Test	Discharge flux (µg/cm²/minute)
AGF pH1.2±0.05	Qusthul Hindi extract SNEDDS	0.436 ± 0.005
	Qusthul Hindi extract solution	0.114 ± 0.007
AIF pH6.8±0.05	Qusthul Hindi extract SNEDDS	0.405 ± 0.007
	Qusthul Hindi extract solution	0.088 ± 0.002

*Data are expressed as mean±SD, n=3

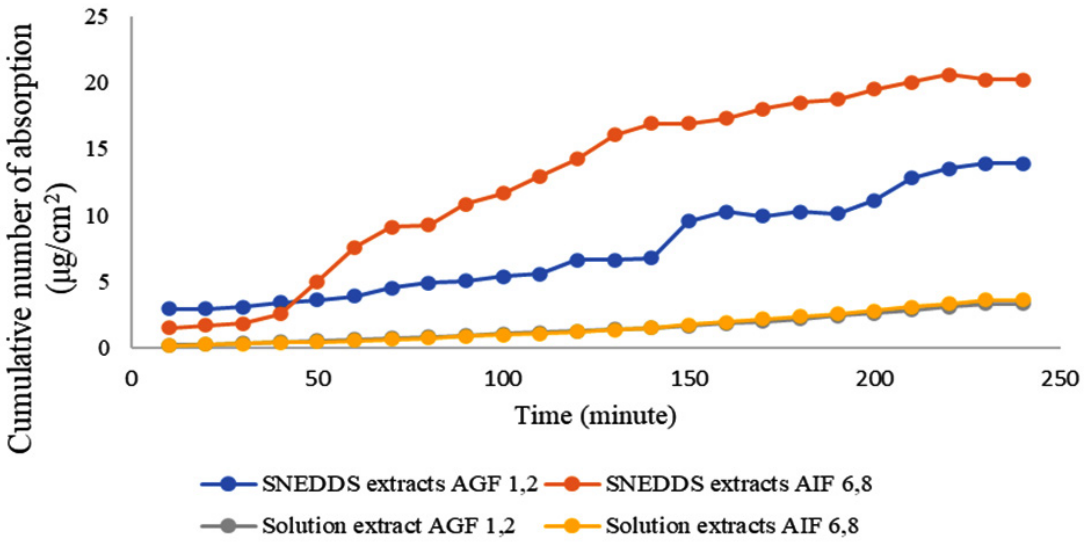

Fig. 2. Graph of the cumulative amount of Qusthul Hindi extract (ig/cm²) absorbed (time vs. the cumulative amount of drug absorbed)

difference between the intestinal permeability of inverted mice to the qustul Hindi extract absorbed in the qustul Hindi extract SNEDDS and the qustul Hindi extract solution in AGF media pH 1.2 ± 0, 05 and AIF pH 6.8 ± 0.05.

DISCUSSION

In the release and absorption test, sampling in the release test and absorption test started at minute 0, and this is because the release test and absorption test in this study used a continuous

Table 4. The cumulative amount of Qusthul hindi extract (ig/cm^2) absorbed (p-value 0.05)

Time (minute)	Cumulative number of SNEDDS extracts Qusthul Hindi absorbed (µg/cm^2)*		Cumulative amount of extract solution Qusthul Hindi absorbed (µg/cm^2)*	
	AGF 1.2 ± 0.05	AIF 6.8 ± 0.2	AGF 1,2 ± 0,05	AIF 6,8 ± 0,2
10	2.874 ± 0.289	1.511 ± 0.166	0.077 ± 0.000	0.077 ±0.000
20	2.898 ± 0.381	1.469 ± 0.078	0.161 ± 0.005	0.146 ± 0.012
30	2.930 ± 0.302	1.667 ± 0.156	0.240 ± 0.018	0.210 ± 0.003
40	3.045 ± 0.545	1.817 ± 0.076	0.320 ± 0.024	0.277 ± 0.019
50	3.408 ± 0.569	2.544 ± 0.179	0.410 ± 0.042	0.354 ± 0.018
60	3.611 ± 0.733	4.955 ± 0.722	0.499 ± 0.047	0.433 ± 0.021
70	3.897 ± 0.423	7.568 ± 0.117	0.591 ± 0.034	0.519 ± 0.019
80	4.522 ± 1.262	9.087 ± 0.407	0.687 ± 0.051	0.620 ± 0.043
90	4.900 ± 1.170	9.267 ± 0.511	0.800 ± 0.048	0.727 ± 0.023
100	5.018 ± 1.030	10.840 ± 0.239	0.910 ± 0.056	0.833 ± 0.047
110	5.339 ± 1.498	11.650 ± 1.117	1.022 ± 0.047	0.947 ± 0.078
120	5.557 ± 1.652	12.942 ± 0.686	1.136 ± 0.046	1.063 ± 0.077
130	6.610 ± 0.195	14.240 ± 0.663	1.254 ± 0.054	1.210 ± 0.066
140	6.592 ± 0.121	16.071 ± 0.352	1.374 ± 0.043	1.345 ± 0.064
150	6.741 ± 0.737	16.901 ± 0.385	1.495 ± 0.082	1.483 ± 0.068
160	9.558 ± 2.443	16.919 ± 1.077	1.642 ± 0.096	1.707 ±0.132
170	10.235 ± 0.912	17.322 ± 0.755	1.795 ± 0.098	1.925 ± 0.120
180	9.918 ± 0.792	18.011 ± 0.306	1.967 ± 0.170	2.124 ± 0.103
190	10.266 ± 0.768	18.512 ± 1.212	2.167 ± 0.093	2.335 ± 0.108
200	10.110 ± 1.887	18.728 ± 0.582	2.365 ± 0.190	2.536 ± 0.083
210	11.121 ± 0.092	19.519 ± 0.859	2.599 ± 0.181	2.768 ± 0.080
220	12.811 ± 4.084	20.026 ± 0.687	2.821 ± 0189	3.041 ± 0.071
230	13.516 ± 0.629	20.630 ± 1.412	3.053 ± 0.184	3.319 ± 0.035
240	13.897 ± 1.715	20.228 ± 0.729	3.303 ± 0.158	3.599 ± 0.060

*Data are expressed as mean±SD, n=3

Table 5. The absorption flux (µg/cm^2/minute) of SNEDDS and Qusthul Hindi extract solution (*Saussurea lappa*)

Media	Test	Absorption flux (µg/cm^2/min)
AGF pH1.2±0.05	Qusthul Hindi extract SNEDDS	0.186 ± 0.002
	Qusthul Hindi extract solution	0.038 ±0.001
AIF pH6.8±0.05	Qusthul Hindi extract SNEDDS	0.298 ± 0.009
	Qusthul Hindi extract solution	0.050 ± 0.003

*Data are expressed as mean±SD, n=3

Table 6. Inverted rat intestinal permeability values for SNEDDS and Qusthul Hindi (S*aussurea lappa*) extract solution (cm/minute)

Media	Test	Inverted rat intestinal permeability to Qusthul hindi extract (cm/minute)
AGF pH 1.2 ±0.05	Qusthul Hindi extract SNEDDS	$1.9 \times 10^{-4} \pm 0.000$
	Qusthul Hindi extract solution	$1.3 \times 10^{-4} \pm 0.000$
AIF pH 6.8 ±0.05	Qusthul Hindi extract SNEDDS	$2.2 \times 10^{-4} \pm 0.000$
	Qusthul Hindi extract solution	$1.0 \times 10^{-4} \pm 0.000$

*Data are expressed as mean±SD, n=3

system so that the process could not be interrupted because the parameters to be measured were to determine the rate. The release and absorption rates are the cumulative amounts released or absorbed. Release and absorption testing is carried out in vitro, which has the main advantage, namely that the experiment can be controlled precisely, and the variables used are only the membrane and the material to be tested. In this method, the solution that will be tested for its dissolution and diffusion capabilities under in vitro conditions must be assumed to be as follows: (1) The receptor phase is in perfect "sink" condition (2) the decrease in concentration in the donor is very small (3) the membrane used is a fragile and flat homogeneous membrane[11]

Hopefully, the results of the in vitro testing in this research can be further developed in vivo testing. Information about in vitro-in vivo correlation (IVIVC) helps develop pharmaceutical dosage forms and new products. Regarding the development of SNEDDS, IVIVC data is needed and must be studied further to accurately predict the desired bioavailability characteristics for a control product release[8]. According to the United States Pharmacopoeia (USP), IVIVC establishes a rational relationship between biological properties or parameters obtained from biological properties produced by a dosage form and the physical-chemical properties or characteristics of the same dosage form[12]. The FDA defines IVIVC as a form of a predictive mathematical model that describes the relationship between the in vitro properties of a dosage form and a relevant in vivo response. In general, in vitro, characteristics are the speed and amount of dissolved or dissolved drug, while the in vivo response is the drug concentration in plasma or the amount of drug absorbed[8].

Research was conducted on the relationship between in vitro and in vivo SNEDDS testing. In his study, he compared in vitro testing using Fick's law equation and in vitro lipolysis with in vivo testing using white mice on the oral absorption of BCS class II drugs (griseofulvin (GRI), phenytoin (PHE), indomethacin (IND), and ketoprofen (KET)). in SNEDDS. The results of in vitro studies show that GRI, PHE, IND, and KET in SNEDDS increase the absorption of SNEDDS 6–8 times. Meanwhile, in vivo, GRI, PHE, IND, and KET absorption increased 15-21 times compared to the suspension form. Therefore, developing SNEDDS with in vitro and in vivo testing can help establish correlations with oral absorption, which may influence the properties of the drug ingredients and oils in SNEDDS[13].

Several supporting studies have proven the controlled release profile of drug compounds using SNEDDS, including research into developing the SNEDDS formula design using Curcumin and Piperine as medicinal ingredients. In this study, releasing qustul Hindi extract in SNEDDS resembles research on Curcumin and Piperine in SNEDDS. Pure curcumin without SNEDDS showed negligible % drug release (H"0%) in both AGF and AIF media. Meanwhile, a significant increase in the release of Curcumin and Piperine ($p < 0.05$) occurred in the self-nanoemulsifying formulation[12]. The release date shows the release of the qustul Hindi extract solution. Several factors influencing drug release include viscosity and chemical bonds of the active ingredient with the carrier. One study of SNEDDS formulations with

the same components but using different methods explained that the bioavailability of cyclosporine (CsA) in mice formed particles of various sizes by administering the drug in a nanoemulsion. By reducing the droplet size, CsA increases oral absorption. The SNEDDS CsA test was carried out on healthy volunteers with characteristic test results of 25-400 nm particle sizes. This study showed an inverse correlation between the particle size of the studied SNEDDS and the oral bioavailability of CsA[12]. Based on the results of this research, it can be concluded that particle size distribution is critical because it affects the availability of drugs in the body after oral administration.

Absorption testing was carried out using a Wilson Crene Tube. The membrane used for the absorption test was the inverted rat intestine. The test conditions were identical, namely at a temperature of 37°C. Sampling is equalized for each test, namely 1 mL. The absorption of the samples was observed using a UV spectrophotometer (Shimadzu UV-1800) at a maximum wavelength of 306 nm. The measured levels were corrected using the Wurster equation. After that, a curve was created between the cumulative amount absorbed and time. The absorption profile of the qustul Hindi extract and the qustul Hindi extract solution on AGF medium pH 1.2 ± 0.05 and AIF pH 6.8 ± 0.05 showed an increase in the cumulative amount of qustul Hindi extract absorbed. This happens to both SNEDDS of qustul Hindi extract and qustul Hindi extract solution, which shows that qustul Hindi extract can be absorbed at a higher rate through the intestine than qustul Hindi given in extract solution form.

Based on the results of the two-way ANOVA statistical analysis on the absorption test, it showed that there was a significant difference between the absorption flux of SNEDDS of qustul Hindi extract and qustul Hindi extract solution on AIF pH 6.8 and AGF 1 media. 2 ($p=0.01$). Therefore, the carrier matrix (SNEDDS) significantly increased the flux and cumulative amount of qustul Hindi extract absorbed through the inverted rat intestine, which was used as a membrane in the absorption test. The diffusely absorbed profile of qustul Hindi extract describes the amount of drug absorbed at a certain time interval.

The absorption process is faster in AIF media pH 6.8 ± 0.2 than in AGF media pH 1.2 ± 0.2. This is probably because the pH 6.8 medium is much more alkaline than the pH 1.2 AGF medium, so the qustul Hindi extract, probably a weak acid, will dissolve more easily. Increasing the acidity of the simulation media fluid will increase the qustul Hindi extract in molecular form so that its solubility becomes lower. The balance of qustul Hindi extract in a molecular and ionized form is better in the AIF medium pH 6.8. This causes the highest absorption of qustul Hindi extract in the AIF medium pH 6.8. Low solubility in water and lack of permeability to penetrate the absorption barrier can affect the bioavailability of a natural compound in the body[12]. Through the SNEDDS absorption mechanism, one of which is the formation of changes in the physical barrier function of the digestive tract because the presence of SNEDDS components causes an increase in permeability. The permeability of biological membranes to a drug can be described by its partition coefficient as a linear relationship with its absorption rate. It was obtained based on the two-way ANOVA statistical analysis of permeability calculations ($p=0.02$). This shows a significant difference between the intestinal permeability of inverted mice and the qustul Hindi extract absorbed in SNEDDS. Linear accumulation, or in other words, there is no change due to changes in time. This discharge follows a discharge order. The release profile obtained based on this research is based on its limited ability to adsorb certain compounds when passing through the gastrointestinal (GI). The application of SNEDDS in oral use shows that with a relatively long period of use, a small dose can be given to obtain optimal results from the drug compound used, thereby reducing the side effects of the drug used.

The release and absorption of SNEDDS from qustul Hindi extract follow zero order kinetics occurring through an interfacial transport mechanism across the surfactant layer coating the emulsion droplets followed by diffusion and convective transport through the surrounding aqueous medium. The release process in the nanoemulsion system occurs because the components that make up the formula dissolve, causing the nanoemulsion to break and the active

substance to be released. The precise kinetic expression depends on (1) whether the drug enters the aqueous phase as a free molecule that then "reacts" with the micelles or enters the aqueous phase by partitioning directly into micelles and (2) the rate-limiting step in interfacial transport, mass transport, or reaction with micelle. To explore drug release influenced by the physical and chemical characteristics of the resulting SNEDDS formula.

CONCLUSION

In conclusion, the selected formulas using Miglyol 812, Tween 80, and PEG 400 components at concentrations of 2.13%, 5.81%, and 2.06% were suitable as SNEDDS formulations. The SNEDDS formula of qusthul Hindi extract provided in vitro release flux results that were 4.20 times higher in AGF media at pH 1.2 ± 0.05 and 5.62 times higher in AIF at pH 6.8 ± 0, 05 compared with a solution of the extract. The In vitro absorption results also indicated a higher increase of 4.02 times in AGF and 3.32 times higher in AIF compared to the extract solution.

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Conflict of Interest

The author(s) do not have any conflict of interest

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Authors' Contribution

Rahmi Annisa: Funding Acquisition, Resources, Supervision; Nabila Rahmadani, Wirda Anggraini: Data Collection, Analysis, Writing – Review & Editing; Avin Ainur Fitrianingsih: Data Collection, Analysis, Writing – Review & Editing; Roihatul Muti'ah: Visualization, Supervision, Project Administration; Begum Fauziyah: Visualization, Supervision, Project Administration.

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