International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Issue 2, 2024

Original Article

MODIFYING FRACTION EXTRACTED FROM SESEWANUA *(CLERODENDRUM FRAGRANS* WILD) LEAVES IN SNEDDS PREPARATIONS: CHARACTERIZATION AND BIOAVAILABILITY TEST

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Received: 14 Sep 2023, Revised and Accepted: 17 Jan 2024

ABSTRACT

Objective: This study aimed to determine the effect of compound modification using acyl chloride derivatives on n-hexane: ethyl acetate fraction of sesewanua leaves, focusing on the characteristics and pharmacokinetics profile in Self-Nano-emulsifying Drug Delivery System (SNEDDS) preparations.

Methods: A quasi-experimental method was used with six SNEDDS formulas, namely F0 (without active substance), F1 (acetyl chloride fraction), F2 (propanoyl chloride fraction), F3 (butyryl chloride fraction), and F4 (pentanoyl chloride fraction) and F5 (piperine compound). The fractions were subjected to characterization tests, including particle size, polydispersity index, and zeta potential as well as determination of pharmacokinetics profile using the modified crane and Wilson method.

Results: The results showed that the characterization tests of particle size using Particle Size Analyzer (PSA) for F0-F5 on gastric fluid included 15.8, 17,367, 20,367, 15.8, 28.233, and 21.533 nm. The polydispersity index values were 0.211, 0.438, 0.311, 0.383, 0.394, and 0.397, while the Zeta Potential values were-22,267,-22.2,-23.5,-24,033,-22,967, and-21.6 mV, respectively. The pharmacokinetics profile of AUC0-∞ was as follows: F0 0 µg, F1 492.83, F2 492.83, F3 245.98, F4 492.94, and F5 843.38 µg. Fraction five (F5) as a control had a higher AUC0-∞ value than compared to the fractions modified with acyl chloride derivatives. The T1/2 elimination values were F0 0 h, F1 22.5 h, F2 10.811 h, F3 35.54 h, F4 231.01 h, and F5 15.469 h.

Conclusion: Based on the results, the addition of acetyl, propanoyl, butyryl, and penthanoyl chloride affected Particle Size Characterization Analysis and pharmacokinetics profile of SNEDDS preparation of n-hexane: ethyl acetate fraction. Structural modification showed the ability to alter the bioavailability of the active ingredient according to the desired therapeutic goal.

Keywords: Particle size analysis, AUC, Nanoparticle, Crane and wilson

DOI: https://dx.doi.org/10.22159/ijap.2024v16i2.49372 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Sesewanua (*Clerodendrum fragrans* Wild.) was reported to have anti-inflammatory activity, based on the study conducted by Sapiun *et al.*, (2023). The emulgel ethanol extract of sesewanua leaves at a dose of 5% had a higher percentage of inflammatory inhibition (34%) compared to the positive control (21%). A total of four compounds have been identified in the ethanol extract of sesewanua leaves, including flavonoids, alkaloids, tannins, and saponins [1]. The isolation of compounds was performed using n-hexane and ethyl acetate solvents. From the fractionation process, the most active fraction was obtained, namely 1 (9:1, 8:2, 7:3, 6:4) with very strong antioxidants, as shown by an IC50 value of 2.56033 mg/l. Based on the results, this fraction contains alkaloids with Rf value of 0.9 [2].

The oral administration of drugs accounts for more than 70% of the total dosage forms. The popularity of this route is attributed to the ease and acceptance by patients, which increases compliance [3]. However, n-hexane: ethyl acetate fraction generally causes discomfort when used orally, resulting in the development of nanotechnology pharmaceutical preparations, namely the Self-Nano-emulsifying Drug Delivery System (SNEDDS).

Formulation of SNEDDS preparations can improve the bioavailability of the active substance through oral use and increase the rate of dissolution as well as absorption in the body [4-6]. SNEDDS fractions of n-hexane: ethyl acetate have also been tested orally and shown to reduce rheumatoid arthritis index values by 44.66%. It also decreased swelling in rat legs induced with Complete Freund's adjuvant (CFA) by 79.25% [7, 8]. Aside from formulating active compounds in SNEDDS preparations, another strategy that can increase the ability of plant-active compounds to achieve maximum therapeutic effects is modifying the structure [9, 10].

Modification of the compound structure can be achieved through an acyl nucleophilic substitution reaction (acylation reaction) [11, 12]. Using acyl chloride derivatives is a method for adding structures to form ester groups that enhance the lipophilicity of compounds. The greater the lipophilicity of a compound, the easier the rate of penetrating the stomach and intestinal membranes [13, 14]. The analgesic efficacy of pinostrobin isolated from cat encounters has been structurally modified using acyl chloride, including acetyl, propanoyl, butyryl, and pentanoyl chloride. At a dose of 14 mg/kg body weight, the percentage of pain resistance from pinostrobin acetate was 46.57%, while those of propionate, butyrate, and pentanoate were 47.33%, 49.2%, and 55.34% respectively. For diclofenac sodium as a positive control, the percentage of pain resistance was 42.37%, and pinostrobin in the form of a negative control amounted to 33.97% [11]. Based on the study by Suryadi *et al.* (2018), structural modifications in a compound can increase the activity characterized by an increasing percentage of pain resistance.

Bioavailability profile is necessary to understand the fate of a drug in the body, and this parameter is influenced by absorption [15, 16]. The parameters used to identify drug bioavailability are Peak Plasma Concentration (Cpmax), Peak Time (T_{max}), half-life (T1/2), and Area Under Curve (AUC0-∞) [17]. Based on the previous description, this study aimed to conduct bioavailability tests of n-hexane: ethyl acetate fraction derived from sesewanua leaves (*Clerodendrum fragrans* Wild) modified using acyl chloride in SNEDDS Preparations.

MATERIALS AND METHODS

Materials

The tools used included watch glass, *Pyrex*® glassware, drip pipette, *ESCO Frontier*® fume hood, stirring rod, column, and beaker glass *pyrex*®, disposable syringe 3 ml *one med*®, *pyrex*® measuring cup, Erlenmeyer *pyrex*®, horn spoon, spatula, magnetic stirrer *Thermo Scientific*®, PSA (*Cilas 1190*), *Mettler Toledo Multi Parameter*®, *Sartorius*® analytical balance, *iwaki*® measuring flask, digital pH meter and UV1900i Spectrophotometry (*Shimadzu*®).

Plant material

Sesewanua leaves (*Clerodendrum fragrans* Wild.) were collected from Dulamayo Village, Bongomeme District, Gorontalo Regency, Gorontalo (0 °36'08.6″ N, 122 °52'03.0″ E). Botanical identity was confirmed at the Research Center for Plant Conservation and Botanic Gardens of the Indonesian Institute of Science (number B-418/IPH.3/KS/II/2019, February 25, 2019. Other materials used were Aquadest (*OneMed*®), acetic acid (*Total Equipment Pharmacy*), butyric acid (*Ilmu Kimia*), hydrochloric acid (*Total Equipment Pharmacy*), pentanoic acid (*Total Equipment Pharmacy*), propanoic acid (*Total Equipment Pharmacy),* Dyalysis Tubing, ethanol 96% (*SAE*®), ethyl acetate (*Total Equipment Pharmacy*), cotton, filter paper, sodium hydroxide (*Total Equipment Pharmacy*), *n*-hexan (*Total Equipment Pharmacy*), PEG 400 (*Total Equipment Pharmacy*), Piperine (*Total Equipment Pharmacy*), silica gel60 (KGaA) (*Chemix Yogyakarta*), Tween 80 (*Total Equipment Pharmacy*), and VCO (*Sucofindo*®).

Ethical approval

Before conducting this study, ethical approval was received from the Ethics Commission of the Health Polytechnic of the Ministry of Health of Gorontalo (No. DP.01.01/KEPK/68/2023.

Fractionation of sesewanua leaves ethanol extract

The separation of ethanol extract to isolate alkaloid group compounds was achieved using column chromatography with n-hexane: ethyl acetate mobile phases in ratios of (9:1), (8:2), (7:3), and (6:4). The first step in the chromatographic process was to insert filter paper and cotton in the lower column. Subsequently, 5 g of cleaned sand was placed inside and recoated with cotton wool. About 20 g of silica gel_{60} was added and mixed with ethyl acetate until the mixture was slurry-shaped. Following this, the extract (10 g) dissolved in ethanol was added to the column and allowed to descend slowly through the silica gels. It was then eluted, starting from a low polarity, and slowly increased. The fraction produced was evaporated and added with 1 g aeroside [2, 18, 19].

Fraction Yield Formula:

$$\text{Yield (\%)} = \frac{\text{Fraction Weight}}{\text{Extract Weight}} \text{x } 100\% \text{ .....[20]}$$

Modification of n-hexane: ethyl acetate compound with acyl chloride derivative

Modification of n-hexane: ethyl acetate compound using acetyl, propionyl, butyryl, and pentanoyl chloride was carried out by drug complexation. Initially, n-hexane: ethyl acetate fraction (0.5 g) was dissolved in 10 ml of 96% ethanol solvent (phase A). About 1 g NaCl as a complexing compound was dissolved in 1 ml each of acetic, propanoic, butyric, and pentanoic acid (phase B). Phases A and B were mixed and evaporated at a temperature of 100 °C until a powder formed [21].

SNEDDS was prepared using the high-pressure homogenization (HPH) method or the method of preparing high-pressure homogenizer nanoemulsions. This method is frequently used for drug degradation of high-molecular-weight compounds. It was carried out using a hot plate with a magnetic stirrer in a gradually emulsified mixture. The initial step entailed mixing the oil phase (VCO) and the active substance (n-hexane: ethyl acetate fraction) with a magnetic stirrer at a speed of 310 rpm for 5 min. Surfactant was added, followed by stirring at the previous speed and duration, and then cosurfactant was added [22–24].

Characterization of SNEDDS

pH

The pH of SNEDDS preparations was measured using a calibrated pH meter [25, 26].

Table 1: SNEDDS dosage modification of n-hexane: ethyl acetate fraction from sesewanua leaves

Materials	Concentration (g)					
	F0	F1	F2	F3	F4	F5
Active Substances	0	0.005	0.005	0.005	0.005	0.005
VCO	1	1	1	1	1	1
Tween 80	7	7	7	7	7	7
PEG 400	2	2	2	2	2	2

F0 = No Active Substance (Negative Control), F1 = Fraction *n*-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua

Nanoemulsified time

Nano-emulsion time testing was carried out using a magnetic stirrer. A total of 1 ml SNEDDS preparation was placed into a container filled with pH 3.5 and stirred with a magnetic stirrer at a speed of 1000 rpm. Observations were made on the time required from the beginning of SNEDDS droplets in the media until nanoemulsions were formed [19, 27].

Analysis using particle size analyzer (PSA)

Particle size, polydispersity index, and zeta potential were identified using PSA Dynamic Light Scattering (DLS) method. The sample was diluted with a dispersing solution, creating a cuvette cell at a volume of 3 ml. The cuvette containing the sample was placed into the cuvette holder inside PSA device. System settings regarding sample name, dispersing media, and gate time were set up using the software. Subsequently, "Start Measurement" was clicked to start the analysis process [25].

SNEDDS bioavailability test

Bioavailability testing of SNEDDS was performed using an artificial crane and the Wilson method [28, 29]. In this test, 2 ml SNEDDS and 5 ml gastric fluid (pH 3.5) were enclosed in the dialysis membrane. The dialysis membrane was created in a beaker filled with 96% ethanol-receiving medium (blood circulation) at a volume of 250 ml. Samples were made at 3 ml each for 24 h with 12 points, and then 4 points that met the peak and steady-state levels were collected. After each sampling, the receiving medium was substituted to maintain sink condition [28, 30].

Spectrometry test using UV-VIS Spectrophotometry

Determination of the maximum wavelength of piperine

Piperine (50 mg) was dissolved in 50 ml ethanol (1000 ppm) and diluted to a concentration of 500 ppm. A 500 ppm solution of piperine was diluted to 1, 5, and 10 ppm, then absorbance was recorded at a wavelength of 200-600 nm.

Piperine standard curve

Piperine weighing 50 mg was dissolved in 50 ml ethanol (1000 ppm) and diluted to a concentration of 500 ppm. Variations in the concentrations of 1, 2, 3, 4, 6, and 10 ppm were determined. Absorbance was measured using a UV-Vis spectrophotometer at λ maximum wavelength (342.50 nm). The absorbance results (y) and the concentration of the piperine raw solution (x) were plotted in a calibration curve to obtain a linear regression equation y=bx±a [31].

Determination of piperine levels

The absorbance (342.50 nm) of each sample was monitored using a UV-Vis spectrophotometer and testing was conducted in triplicate.

Bioavailability parameters were obtained using the formulas below [17, 32]:

$AUC_{0-\infty}$ was calculated by the trapezoid method,

$$AUC_{0-\infty} = \sum_{n=1}^{\infty} \left(\frac{(Cp1+Cp2)}{2} x\ (t2 - t1) \right) [17]$$

Cp_{max}= Maximum absorbed concentration

T_{max}= The time to reach maximum concentration

$T_{1/2}$= 0,693

Data analysis

Bioavailability data of $T_{1/2}$ and $AUC_{0-\infty}$ were statistically analyzed using *Statistical Package for the Social Sciences* (SPSS) program version 25.0. To determine the normality of the data, the Shapiro-Wilk test was performed. The results with a p-value<0.05 were considered abnormally distributed. The Homogeneity of variance test obtained a value of>0.05, meaning that the data distribution was not homogeneous (different data variations). One-way ANOVA Post hoc *Tamhane's T2* test obtained significant scores.

RESULTS AND DISCUSSION

Yield percentage of n-hexane: ethyl acetate fraction from sesewanua leaves

As shown in table 2, the percent yield of n-hexane: ethyl acetate fraction had an excellent value, in line with the Indonesian Herbal Pharmacopoeia, which set the limit of a good extract yield at 7.2% [33]. The higher the yield of the extract fraction, the greater the amount of compounds extracted between solvents [34]. This was attributed to the nonpolar nature of n-hexane solvent as well as the semipolar nature of ethyl acetate, causing the withdrawal of the compound from n-hexane: ethyl acetate fraction [35].

Table 2: Yield percent of n-hexane: ethyl acetate fraction

Sample	Solvent	Extract Weight (g)	Fraction Weight (g)	Yield (%)
Sesewanua Leaves	*n*-hexane: ethyl acetate	10	5.2	50.2

Table 3: SNEDDS dosage pH test results

Treatment	SNEDDS pH					
	F0	F1	F2	F3	F4	F5
1	7.7	7.9	8.0	8.0	8.0	8.1
2	8.0	8.0	7.9	8.0	7.7	7.8
3	7.9	7.9	7.8	7.8	7.8	7.9
Mean+SD	7.87+0.15	7.93+0.06	7.90+0.10	7.93+0.12	7.83+0.15	7.93+0.15

F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua, Description: Data represents mean±SD (n=3)

As stated by Ayuningtyas *et al.*, (2021), pH of SNEDDS preparations ranges from 6.5-9.0, while Aisy *et al.*, (2021) reported pH of oral preparations to be 5-8. This implies that the pH of SNEDDS preparations F0, F1, F2, F3, F4, and F5 in table 3 meet pH requirements both for SNEDDS and oral preparations. In addition, alkaloid compounds (piperine) contained in the fraction remained unaffected because SNEDDS preparations were at a stable pH>7 (alkaline) [38]. This was attributed to the addition of surfactants and co-surfactants, which maintained the stability of the active substance [19].

Nanoemulsified time test

Nanoemulsified time test was performed to determine the ease with which SNEDDS formed emulsions while in the body [39]. The test was carried out using gastric (pH 3.5) and intestinal fluid (pH 6.6) [40]. The results of the nano-emulsified time test for SNEDDS preparation are shown in table 4.

SNEDDS emulsified time test results in table 4 meet the standard, namely less than 1 minute. Based on the results, SNEDDS was categorized as grade A, referring to a clear nanoemulsion formed quickly in 1 minute. Clear emulsions with fast emulsified times showed that SNEDDS formulas achieved emulsification easily [27, 36, 37, 41].

PSA analysis

Particle size of SNEDDS

Particle size is a parameter to indicate the characterization of nanoparticles in a drug delivery system [42], and the results obtained in this study are presented in table 5.

Table 4: SNEDDS nano emulated time test results

Treatment	Test nano-emulsified time (Seconds)					
	F0	F1	F2	F3	F4	F5
Gastric fluid	06.42+0.006	04.75+0.006	06.25+0.012	04.87+0.012	04.72+0.006	06.30+0.115
Intestinal fluid	05.81+0.029	05.47+0.012	05.05+0.023	05.68+0.115	05.46+0.023	05.77+0.029

F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua, Description: Data represents mean±SD (n=3)

Table 5: Particle size test results for SNEDDS preparations

Treatment	Particle size (nm)					
	F0	F1	F2	F3	F4	F5
1	16.6	19.5	19.4	16.7	25	22.9
2	15.1	15	21.6	14.9	29.6	21.5
3	15.7	17.6	20.1	15.8	30.1	20.2
Mean+SD	15.8+0.75	17.367+2.26	20.367+1.12	15.8+0.9	28.233+2.81	21.533+1.35

F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua, Description: Data represents mean±SD (n=3)

Based on table 5, the particle size test results using PSA met the acceptable SNEDDS characteristics, namely 5–100 nm in the gastrointestinal tract [43]. This was consistent with a study by Mathew (2022), stating that Quartcetin SNEDDS obtained particle sizes less than 100 [44].

Polydispersity index

Polydispersity index value close to 0 shows homogeneous particle size dispersion, while values above 0.5 imply high heterogeneity [45]. The results for the polydispersity index are shown in table 6.

Table 6: Polydispersity index test results

Treatment	Polydispersity index					
	F0	F1	F2	F3	F4	F5
1	0.359	0.435	0.18	0.361	0.444	0.321
2	0.115	0.449	0.232	0.353	0.473	0.517
3	0.158	0.429	0.52	0.436	0.264	0.352
Mean+SD	0.211+0.13	0.438+0.01	0.311+0.18	0.383+0.05	0.394+0.11	0.397+0.11

F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua, Description: Data represents mean±SD (n=3)

Based on table 6, polydispersity index of SNEDDS preparations was in the appropriate category. Polydispersity index shows the uniformity of globule size in preparation and estimates the range of particle size distribution present in a sample [45].

Zeta potential

The results of the zeta potential test are shown in table 7.

A high zeta potential value is required in a formulation (positive or negative) because it shows the system stability. A good zeta potential value for emulsion preparations ranges between 20-30 mV [46]. The zeta potential value in table 7 complies with the standard.

SNEDDS bioavailability test results

Determination of the piperine standard curve

Measurement of the standard absorbance for piperine is depicted in fig. 1.

The calibration curve in fig. 1 shows the relationship between piperine concentration and absorbance, producing a regression equation to calculate alkaloid levels in the test sample. The regression equation for the piperine standard curve was y = 0.1014x+0.0404 with an r^2 value of 0.9934. An r^2 value close to 1 in the linear calibration curve shows a relationship between the concentration of the piperine solution and the absorbance value.

Concentration and bioavailability test results

Bioavailability shows the speed and amount of active substances that reach the systemic circulation. In this study, bioavailability testing of SNEDDS focused on parameters such as Cp_{max}, T_{max}, $T_{1/2}$, and $AUC_{0-\infty}$ [17]. To determine the bioavailability of SNEDDS preparations, piperine levels (fig. 2) Which successfully penetrated the gastric membrane, were measured.

Bioavailability parameters namely Cp_{max}, T_{max}, $T_{1/2}$, and $AUC_{0-\infty}$ were determined by a curve of piperine levels over time (fig. 2). Cp_{max} refers to the maximum concentration of oral drug administration. Peak plasma levels offer insights into the pharmacological response dependent on drug levels in the body. The higher the plasma levels of the drug in the body, the better the pharmacological response [17,32]. Cp_{max} was identified as the highest peak of each formula on the curve in fig. 2, accounting for maximum concentration of SNEDDS in the body. Cp_{max} and T_{max} values of SNEDDS preparations are presented in table 8.

Table 7: Potential zeta value test results

Treatment	Zeta potential (mV)					
	F0	F1	F2	F3	F4	F5
1	-22.2	-21.8	-23.4	-24.4	-23.4	-21.6
2	-22	-22.8	-22.6	-24.1	-23.3	-22
3	-22.6	-22	-24.5	-23.6	-22.2	-21.2
Mean+SD	-22.267+0.31	-22.2+0.53	-23.5+0.95	-24.033+0.4	-22.967+0.67	-21.6+0.4

F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua, Description: Data represents mean±SD (n=3)

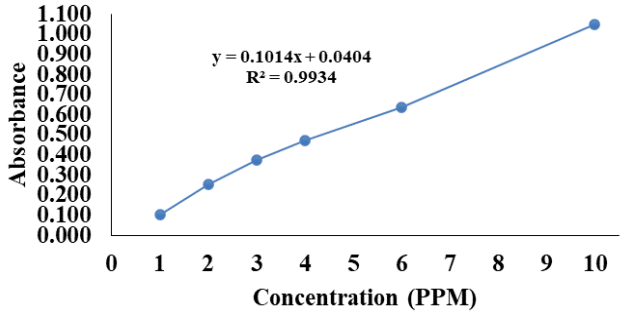


Fig. 1: Piperine standard curve at wavelength 342.50 nm. Each measurement was performed in triplicate (n=3) and data represents mean±SD

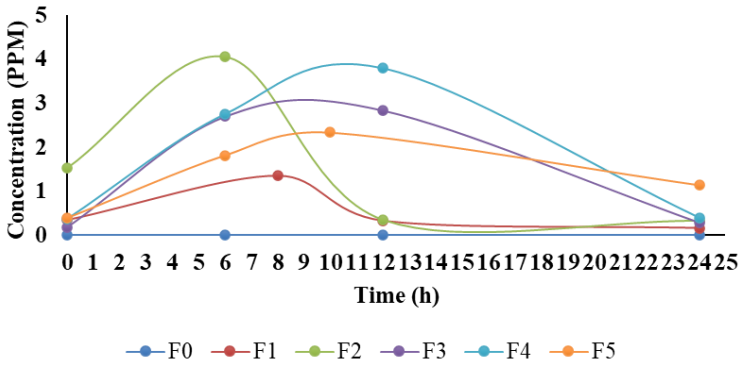
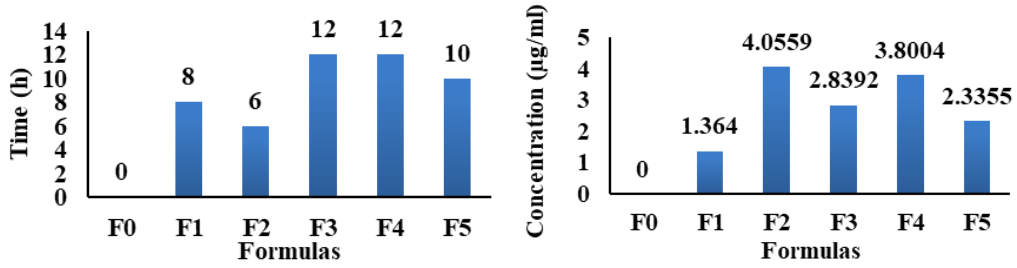


Fig. 2: Profile of piperine levels in the test time interval of 0-24 h in the body. Each value was triplicate (n=3) and data represents mean+SD. F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = n-Hexane: Ethyl Acetate Fraction from Sesewanua leaves

Table 8Bioavailability test of Cp $_{max}$ and T $_{max}$

Test parameters	Bioavailability test					
	F0	F1	F2	F3	F4	F5
Cp $_{max}$ (µg/ml)	0	1,364	4,056	2,839	3,800	2,336
T $_{max}$ (h)	0	8	6	12	12	10

F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua



(A) **(B)**

Fig. 3: (A) SNEDDS Cp$_{max}$ value; (B) SNEDDS T$_{max}$ value. F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction *n*-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua

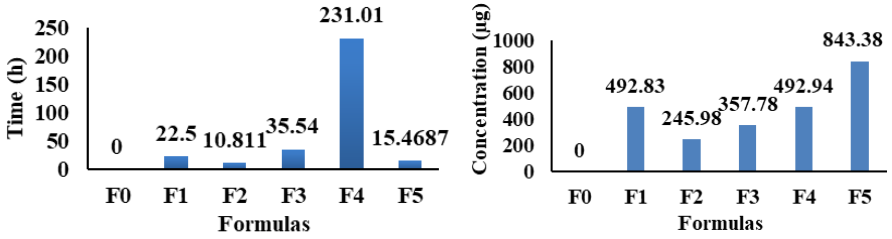
Cp$_{max}$ is associated with T$_{max}$, which refers to the time required for the drug to reach maximum levels in plasma after administration. As stated in previous studies, absorption continues even after reaching the T$_{max}$ value, but the rate becomes slower [17, 32]. Based on fig. 3 (A) and (B), formula 2 has the highest Cp$_{max}$ in a short time, namely 6 h. This was attributed to the more acidic pKa of propanoyl chloride (2.86), making the piperine compound with a pKa of 12.22 more acidic. Meanwhile, the pKa of acetyl, butyryl, and pentanoyl is 4, 4.5, and 3.46 respectively [47,48]. From the principle of "like dissolves like", a compound will dissolve in a solvent with the same properties, meaning that acidic compounds will be more soluble in acidic compounds [49].

The results in fig. 3A and 3B cannot describe the total number of active compounds capable of providing therapeutic effects for the body. The Cp$_{max}$ and T$_{max}$ values can be used as an initial step in determining the pharmacokinetics profile of a drug, including AUC$_{0-\infty}$ and T$_{1/2}$ elimination [50]. The results of AUC$_{0-\infty}$ and T$_{1/2}$ elimination are presented in table 9.

Table 9: Bioavailability test results of AUC $_{0-\infty}$ and T $_{1/2}$ elimination

Test parameters	Bioavailability test					
	F0	F1	F2	F3	F4	F5
AUC$_{0-\infty}$ (µg)	0*	492.83 *	245.98 *	357.78*	492.94*	843.38
T $_{1/2}$ elimination (h)	0*	22.5*	10,811*	35.54*	231.01*	15,469

*Significantly different value against F5 (level 0.05), F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua



(A) **(B)**

Fig. 4: (A) $AUC_{0-\infty}$ SNEDDS value; (B) SNEDDS elimination $T_{1/2}$ value. F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua

$AUC_{0-\infty}$ represents the area under the concentration versus time curve, in other words, it describes the amount of drug absorbed into the system [17, 32, 51]. The results of $AUC_{0-\infty}$ value are presented in fig. 4A. Another bioavailability parameter associated with drug levels in the body is $T_{1/2}$ elimination. $T_{1/2}$ refers to the time required for drug concentrations to be eliminated by half [52,53]. The results of the elimination half-life test are presented in fig. 4B.

Fig. 4A shows the value of $AUC_{0-\infty}$ obtained for n-hexane: ethyl acetate fraction with the addition of acyl chloride derivatives in the order pentanoyl>acetyl>butyryl>propanoyl chloride. Generally, the addition of acyl derivative compounds led to a change in the polarity of piperine. The addition of pentanoyl chloride caused the non-polar piperine to become even more non-polar because pentanoyl chloride has more carbon groups compared to other acyl derivatives. Non-polar compounds tend to easily penetrate the stomach membrane which is non-polar [47, 54]. This is attributed to the chemical bond between pentanoic acid in fat, which is formed through the reaction between the hydroxyl group on glycerol and the carboxyl group on pentanoic acid [55]. The non-polar bond that occurs between pentanoyl chloride and piperine makes the drug compound more non-polar leading to the accumulation for a long time in the body with a slow rate of excretion [56]. Consequently, piperine with pentanoyl chloride derivatives has a very long elimination $T_{1/2}$, namely 231.01 h. The highest $AUC_{0-\infty}$ concentration was observed after the introduction of a new piperine pentanoate derivative.

The addition of acetyl chloride led to changes in the acidity level of piperine, resulting in polarity. However, this change did not completely replace the polarity of piperine. As stated in previous studies, pKa of piperine and acetyl chloride is 12.22 and 4, respectively [47, 48]. Similar to pentanoyl chloride, piperine in acetyl chloride will penetrate the stomach membrane more easily, but at a lower rate. The ability of compounds to become absorbed in body tissues is reduced because there is no direct bond between fat and acetyl chloride [55]. This results in fewer acetyl chloride derivative compounds being absorbed in the body and not accumulating for an extended period due to the reduced polarity of piperine, leading to a short $T_{1/2}$ elimination time. The highest $AUC_{0-\infty}$ concentration was observed after the introduction of butyryl chloride derivatives.

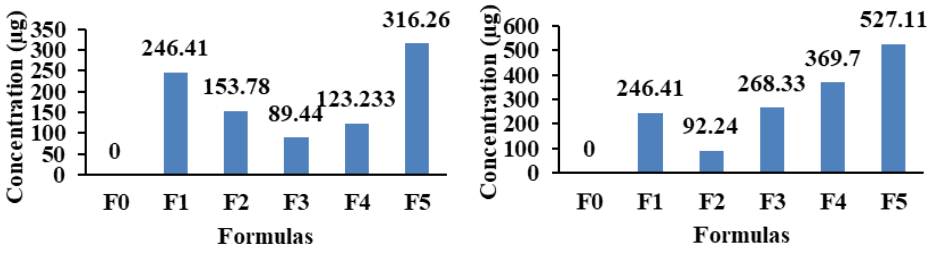
The addition of butyryl chloride causes piperine to change its polarity and become less polar, leading to fewer compounds penetrating the stomach membrane. Additionally, once in fat tissue, the piperine butyryl chloride derivative does not accumulate in the body for an extended period. Similar to acetyl chloride, butyryl chloride does not form direct bonds with fat and the polar nature of this compound translates to a short $T_{1/2}$ elimination time [47, 55]. The lowest $AUC_{0-\infty}$ concentration was observed in piperine with propanoyl chloride derivatives.

The binding of propanoyl chloride with piperine also increases polarity, primarily due to the fewer carbon groups in propanoyl chloride and a more acidic pKa value of 2.86. This results in a lower piperine propanoyl chloride derivative penetrating the non-polar stomach membrane. Moreover, propanoyl chloride cannot bind directly to fat in the body, and the polar nature leads to rapid excretion from the body [47, 48, 55].

The value of $AUC_{0-\infty}$ from piperine without any additions was better compared to when four acyl derivatives were added. This discrepancy can be attributed to the inherent hydrophobic or non-polar properties of piperine, restricting the solubility. Piperine in SNEDDS preparations can increase solubility because the drug dissolves in the oil phase which then forms a nanoemulsion when in contact with water in the digestive system [47, 57]. Due to the non-polar nature, piperine effectively penetrates the non-polar stomach membrane.

Statistical test results for $AUC_{0-\infty}$ and $T_{1/2}$ elimination of SNEDDS piperine compounds showed that there was a significant difference in the values of F0, F1, F2, F3, and F4 compared to F5. Specifically, $AUC_{0-\infty}$ of piperine compounds was significantly influenced by the addition of acetyl, propanoyl, butyryl, and pentanoyl chloride.

Based on $AUC_{0-\infty}$ and $T_{1/2}$ elimination (Figs. 4A and 4B) as well as T_{max} values (fig. 3B), the cumulative concentrations of therapeutic doses and drug concentration during the steady state of SNEDDS preparations were calculated. The concentration value of therapeutic dose and steady-state is shown in fig. 5.



(A) **(B)**

Fig. 1: (A) Drug therapy dosage; (B) Drug concentration in *steady state.* F0 = No Active Substance (Negative Control), F1 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Acetyl chloride, F2 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Propanoyl chloride, F3 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Butyryl chloride, F4 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua+Pentanoyl chloride, F5 = Fraction n-Hexane: Ethyl Acetate leaves Sesewanua

Therapeutic dose is the amount/measure of medication provided to obtain a healing effect, reflecting the drug level in the systemic circulation before reaching $T_{1/2}$ elimination. Meanwhile, a steady state is a condition where drug usage starts to decline slowly and stabilizes when half of the dosage has been eliminated from the blood [58,59]. The pharmacokinetics profile of individual SNEDDS preparations can be obtained through $AUC_{0-\infty}$, $T_{1/2}$ elimination, therapeutic dose, and drug levels during steady state.

Based on fig. 4A and 4B, formula 4 had the highest $AUC_{0-\infty}$ value (492.94 µg) with a very long elimination $T_{1/2}$ value (231.01 h), a high therapeutic dose (369.7 µg) and low steady state (122.233 µg). Therefore, formula 4, containing piperine compound with penthanoyl chloride derivative, can be administrated once every 9-10 d. Formula 1 had a high $AUC_{0-\infty}$ (492.83 µg) short $T_{1/2}$ (22.5 h), a high therapeutic dose (246.41 µg), and steady-state (246.41 µg). This formula, consisting of piperine compounds with acetyl chloride derivatives, can be consumed once a day. Formula 3 had a fairly high $AUC_{0-\infty}$ (357.78 µg) with a slightly long $T_{1/2}$ value (35.54 h), therapeutic dose (268.33 µg), and slightly high steady-state (89.44 µg). Consequently, formula 3, containing piperine compounds with butyryl chloride derivatives can be used once time a day. Formula 2 had a very low $AUC_{0-\infty}$ (245.92 µg), rapid elimination $T_{1/2}$ value (10.811 h), small therapeutic dose (92.24 µg), and steady-state (153.78 µg). This formula containing piperine compounds with propanoyl chloride derivatives can be consumed twice a day. Formula 5 had a high $AUC_{0-\infty}$ (843.38 µg), short elimination $T_{1/2}$ value (15.4687 h), high therapeutic dose (527.11 µg), and elevated steady-state (316.26 µg). Therefore, formula 5 comprising only the piperine compound can be used twice a day. These results were in line with the study by Quader (2017) on SNEDDS and inclusion complexation with hydroxypropyl b cyclodextrin (HPbCD) for enhanced intestinal absorption of eprosartan mesylate [60].

CONCLUSION

In conclusion, the addition of acetyl, propanoyl, butyryl, and pentanoyl chloride significantly affected the pharmacokinetics profile, specifically $AUC_{0-\infty}$ and $T_{1/2}$ elimination of n-hexane: ethyl acetate fraction with antirheumatic effect. Structural modification provided a means to alter bioavailability of the active ingredient according to the desired therapeutic goal. However, this study was conducted on an *in vitro* laboratory scale, showing the need for *in vivo* studies and clinical trials to further prove the results.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Zulfiayu Sapiun contributed to Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing–review and editing, Arlan K. Imran Methodology, Project administration, Supervision, Writing–review and editing, Ahmad Aswad Validation, Writing–original draft, Writing–review and editing, Mohamad Usman Nur Resources, Project administration, Software, Writing–review and editing, Ysrafil Ysrafil Validation, Visualization, Writing–review and editing, Nur'ainun Panigoro Investigation, Methodology, Writing–original draft, Writing–review and editing, Nurrahmatia Utina Investigation, Methodology, Writing–review and editing, and Iksandi Aliwu Investigation, Methodology, Writing–review and editing.

CONFLICT OF INTERESTS

Declared none

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