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BIOAVAILABILITY TESTING OF ASIATICOSIDE FROM ETHANOLIC EXTRACT OF PEGAGAN (*Centela asiatica*) LEAVES SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEMS (SNEDDS) IN WISTAR RATS (*Ratus norvegicus*)

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ABSTRAK

Asiaticoside merupakan senyawa bioaktif dari *Centella asiatica* yang memiliki bioavailabilitas oral rendah akibat kelarutannya yang rendah dalam air. Penelitian ini bertujuan mengevaluasi pengaruh self-nanoemulsifying drug delivery system (SNEDDS) terhadap profil farmakokinetik dan bioavailabilitas oral asiaticoside dibandingkan dengan ekstrak etanol *C. asiatica* konvensional. Metode HPLC divalidasi untuk penentuan asiaticoside dalam serum tikus berdasarkan parameter selektivitas, linearitas, sensitivitas, akurasi, dan presisi. Tikus Wistar diberikan asiaticoside dari ekstrak etanol atau formulasi SNEDDS secara oral, kemudian sampel darah dikumpulkan pada interval waktu yang telah ditentukan hingga 360 menit. Parameter farmakokinetik meliputi Cmax, Tmax, $AUC_{0–t}$, dan bioavailabilitas relatif kemudian ditentukan. Metode HPLC menunjukkan linearitas yang baik pada rentang 0,39–6,25 µg/mL dengan nilai R^2 sebesar 0,9995 serta akurasi dan presisi yang memenuhi kriteria. SNEDDS meningkatkan Cmax dari 0,02362 ± 0,00207 menjadi 0,15374 ± 0,00956 µg/mL dan mempercepat Tmax dari 240 menjadi 120 menit. Nilai $AUC_{0–t}$ meningkat dari 5,22352 ± 0,16074 menjadi 20,8248 ± 0,52093 µg·menit/mL, dengan bioavailabilitas relatif sebesar 398,68% atau sekitar 3,99 kali lebih tinggi dibandingkan ekstrak. Kebaruan penelitian ini terletak pada pembuktian peningkatan absorpsi asiaticoside melalui formulasi SNEDDS. Hasil penelitian memberikan dasar bagi pengembangan sistem penghantaran berbasis nanoemulsi untuk meningkatkan penghantaran oral senyawa bioaktif dengan kelarutan air rendah.

Kata kunci: Asiaticoside, Bioavailabilitas oral, *Centella asiatica* , Farmakokinetik, HPLC, SNEDDS.

ABSTRACT

Asiaticoside is a bioactive compound from Centella asiatica with limited oral bioavailability due to its poor aqueous solubility. This study aimed to evaluate the effect of a self-nanoemulsifying drug delivery system (SNEDDS) on the pharmacokinetic profile and oral bioavailability from ethanolic extract of of C. asiaticoside compared with conventional C. asiatica ethanolic extract. An HPLC method was validated for asiaticoside determination in rat serum based on selectivity, linearity, sensitivity, accuracy, and precision. Wistar rats were administered

asiaticoside from ethanolic extract or SNEDDS orally, and blood samples were collected at predetermined intervals up to 360 minutes. Pharmacokinetic parameters including Cmax, Tmax, AUC_0–t, and relative bioavailability were subsequently determined. The HPLC method showed good linearity over 0.39–6.25 µg/mL with an R^2 of 0.9995 and acceptable accuracy and precision. SNEDDS increased the Cmax from 0.02362 ± 0.00207 to 0.15374 ± 0.00956 µg/mL and reduced Tmax from 240 to 120 minutes. The AUC_0–t increased from 5.22352 ± 0.16074 to 20.8248 ± 0.52093 µg·min/mL, resulting in 398.68% relative bioavailability, approximately 3.99-fold higher than the extract. The novelty of this study lies in demonstrating the substantial enhancement of asiaticoside absorption through SNEDDS. These findings provide a basis for developing nanoemulsion-based delivery systems to improve the oral delivery of poorly water-soluble bioactive compounds.

Keywords: *Asiaticoside, Oral bioavailability, Centella asiatica, Pharmacokinetics, HPLC, SNEDDS.*

INTRODUCTION

Centella asiatica is widely distributed in tropical and subtropical regions and is known by various names in different countries. In India, the plant is known as *Mandukaparni*, while in Western countries it is more commonly known as *Gotu Kola*, a name originating from Sri Lanka (Gray et al., 2018). This plant exhibits various pharmacological properties, which are primarily attributed to its phytochemical constituents, including triterpenes, one of which is asiaticoside (KK, 2020). Asiaticoside can lower fasting blood sugar levels and increase insulin levels (Fitrianda et al., 2017). These effects are related to its role in regulating carbohydrate metabolism enzymes and insulin-related genes, suggesting that asiaticoside has the potential to be developed as a natural antidiabetic agent.

Based on a study by Anukunwithaya et al., (2017), the blood concentration of asiaticoside following oral administration reached only 0.26% bioavailability and an AUC(0 24) value of 2,831 µgh/L. This low bioavailability is impacted by asiaticoside's large molecular size (959.12 g/mol), its hydrophilic nature with a Log P value of −1.03, and a high number of hydrogen bonds (12 donors and 19 acceptors) (Anjelina et al., 2025). Additionally, its low solubility (250 µg/mL) results in low oral absorption and bioavailability (Wardiyah et al., 2024). According to the Biopharmaceutics Classification System (BCS), compounds with low solubility and low permeability are classified as Class IV; therefore, based on these characteristics, asiaticoside is classified as BCS Class IV (Buya et al., 2020).

Low aqueous solubility of an active

substance can pose a challenge for oral delivery. One approach involves utilizing an oil phase to solubilize the active substance leveraging its affinity for the oil followed by the use of surfactants and co-surfactants to facilitate nanoemulsion formation upon contact with water (Wikantyasning et al., 2023). According to a study conducted by Zhao et al., (2015), the use of lipid-based drug delivery systems, such as Self-Nanoemulsifying Drug Delivery Systems (SNEDDS), can increase the bioavailability of compounds with low solubility. Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) consist of a mixture of lipids, surfactants, and co-surfactants that form nanoemulsions in the gastrointestinal tract, thereby enhancing the dissolution and permeation of poorly soluble compounds such as asiaticoside (Zhao et al., 2015). Additionally, SNEDDS can be used to enhance drug transport through the lymphatic system (Cherniakov et al., 2015). This mechanism not only improves the solubility of poorly soluble compounds but also influences the drug absorption pathway in the gastrointestinal tract, allowing more of the drug to reach the systemic circulation. Thus, the use of lipid-based delivery systems such as SNEDDS has the potential to bypass the portal vein route to the liver and increase the oral bioavailability of the drug (Cherniakov et al., 2015).

Bioavailability testing in this study was conducted to determine whether the Self Nanoemulsifying Drug Delivery System (SNEDDS) formulation can increase the bioavailability of asiaticoside from an ethanolic extract of pegagan (*C. asiatica*) leaves in white rats (*Rattus norvegicus*) compared to a non-SNEDDS formulation, in order to assess the extent to which this formulation can enhance bioavailability.

RESEARCH METHOD

Research Design

This study was an experimental study using Wistar rats as animal models. The aim of this study was to determine whether a Self-Nanoemulsifying Drug Delivery System (SNEDDS) formulation can increase the oral bioavailability of asiaticoside from an ethanol extract of gotu kola (*C. asiatica*) leaves compared to administration of the extract without the SNEDDS formulation. This study was conducted at the Faculty of Pharmacy, Muhammadiyah University of Surakarta. The research stages included the preparation of the ethanol extract, the formulation of the SNEDDS, administration of the test preparations to the animals, blood collection at predetermined time intervals, serum preparation, and determination of asiaticoside levels using high-performance liquid chromatography (HPLC). The

asiaticoside concentration time data were then used to describe the pharmacokinetic profile using the parameters AUC, Cmax, and Tmax, followed by a comparison of the relative bioavailability between the two formulations.

Research Target

This study utilized 10 male wistar rats (*Rattus norvegicus*) aged 8–12 weeks weighing 200–300 g. The sample size of 5 animals per group was justified based on the Resource Equation Method calculation (E = N - B, where E = 10 - 2 = 8, approaching the ideal range of 10–20 for exploratory studies) and the application of the Reduction principle from the international CIOMS/WHO guidelines to minimize animal use without compromising statistical validity. All animals were allocated via arbitrary randomization into two treatment groups (n = 5) the asiaticoside ethanolic extract of *Centella asiatica* leaves group (non-SNEDDS) and the asiaticoside extract in SNEDDS formulation group, with separate sample handling and coding for each group to control bias and prevent cross-contamination. This study protocol was approved by the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Universitas Muhammadiyah Surakarta, under Ethical Clearance Certificate No. 6052/A.1/KEPK-FKUMS/XII/2025 dated December 15, 2025, in compliance with the Declaration of Helsinki, CIOMS, and WHO 2016 standards.

Preparation of Ethanolic Extract from *Centella Asiatica* Leaves

C. asiatica leaves were dried in an oven at 35–40°C for two days, then ground in a blender to produce a crude powder. The extraction process was carried out by soaking 250 g of the crude powder in 2.5 L of 96% ethanol at a solid-to-solvent ratio of 1:10. The mixture was re-soaked after three days, then filtered using filter paper and a Buchner funnel. The resulting filtrate was then evaporated using a rotary evaporator to obtain a concentrated extract (Melati & Setiyadi, 2025).

Preparation of Self-Nanoemulsifying Drug Delivery Systems

Self-Nanoemulsifying Drug Delivery Systems was prepared using a ratio of soybean oil; Tween 20; PEG 400 = 2.5; 2; 0.5 (v/v). A 200 mg ethanol extract of gotu kola leaves was added to 5 mL of the mixture, vortexed for 5 minutes, sonicated for 5 minutes, and then incubated for 5 minutes at 45°C; this procedure was repeated three times (Melati & Setiyadi, 2025).

Determination of Asiaticoside Content in Extracts and SNEDDS

The asiaticoside content was determined using High Performance Liquid

Chromatography (HPLC). A standard solution of asiaticoside was prepared by dissolving 5 mg of the asiaticoside standard in 500 µL of dimethyl sulfoxide (DMSO) until homogeneous, yielding a stock solution with a concentration of 10 mg/mL (Chemical, 2022), a series of concentrations (0.39, 0.781, 1.56, 3.125, and 6.25 µg/mL) was then prepared to create a calibration curve. The analysis was performed using an RP-C18 column (250 × 4.6 mm; 5 µm) with an isocratic mobile phase of acetonitrile and water (60:40, v/v), a flow rate of 1.0 mL/minute, and detection using a UV detector at a wavelength of 214 nm (Madhusudhan et al., 2014). 100-mg sample of the ethanol extract of gotu kola leaves was weighed, then dissolved in acetonitrile:water mobile phase (60:40, v/v) to a final volume of 10 mL and sonicated for 10–15 minutes to ensure complete dissolution. The solution was then filtered using a 0.22 µm PTFE membrane before being injected into the HPLC system (Melati & Setiyadi, 2025). Three milimeter of SNEDDS was centrifuged at 3000 rpm for 20 minutes to separate the pellet from the supernatant. The supernatant was separated from the pellet, and 1 mL of the supernatant was transferred to a 10 mL volumetric flask and diluted with acetonitrile:water mobile phase (60:40, v/v) to the mark (Melati & Setiyadi, 2025). Subsequently, 10 µL volume of each sample was then injected into the High-Performance Liquid Chromatography (HPLC) system (Anukunwithaya et al., 2017).

Method Validation

Method validation was performed using spiked serum samples injected into the HPLC system. A 50 µL aliquot of the serum was mixed with 200 µL of an acetonitrile:water (60:40, v/v) mobile phase and vortexed until homogeneous. The mixture was then centrifuged at 10,000 rpm for 10 minutes and filtered through a 0.22 µm PTFE membrane prior to injection into the HPLC system (Anukunwithaya et al., 2017). The prepared spiked serum served as the sample for the bioanalytical method validation process, conducted in accordance with the Food and Drug Administration (FDA) Bioanalytical Method Validation Guidance (2018); the parameters evaluated included selectivity, sensitivity, linearity, accuracy, and precision. Selectivity was assessed using 6 blank serum samples and 6 blank serum samples spiked with asiaticoside at the Lower Limit of Quantification (LLOQ) concentration of 0.39 µg/mL. Sensitivity was determined through LLOQ testing with five replicates. Linearity was evaluated using five asiaticoside standard concentrations: 0.39, 0.781, 1.562, 3.125, and 6.25 µg/mL.

Accuracy and precision testing involved four Quality Control (QC) levels LLOQ (0.39 µg/mL), Low Quality Control (LQC; 1.17 µg/mL), Medium Quality Control (MQC; 3.125 µg/mL), and High Quality Control (HQC; 5.00 µg/mL) each analyzed in five replicates. Results were evaluated based on the coefficient of determination (R^2), Coefficient of Variation (%CV), and percentage recovery value, in accordance with FDA acceptance criteria (Food and Drug Administration, 2018).

Bioavailability Testing

Wistar rats were divided into two treatment groups, the first group was administered 2.5 mL of ethanolic extract of pegagan leaves, and the second group was administered 2.5 mL of ethanolic extract of pegagan leaves in SNEDDS formulation. Prior to treatment, the preparations were adjusted to an equivalent asiaticoside concentration of 10 µg per 2.5 mL volume, wherein the SNEDDS formulation contained 10 µg asiaticoside per 2.5 mL, and the extract group utilized 37.57 mg of extract (equivalent to 10 µg asiaticoside) reconstituted in 2.5 mL distilled water. Prior to administration, the rats were fasted for 12 hours to avoid food-drug interactions, but were provided free access to drinking water (Kazi et al., 2019). After oral administration, 400 µL of blood was collected from the retrobulbar orbital vein using a sterile glass tube at time points of 0, 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes using a safe physical restraint technique without anesthesia. Immediately following each blood sampling, fluid rehydration was administered via oral gavage with 1mL of 0.9% NaCl solution. Throughout the testing period, the physiological condition and welfare of the animals were monitored periodically by observing respiratory rate, physical activity, and motor response to ensure the rats experienced no severe distress. The collected blood was transferred to 1,5 mL microtubes and then centrifuged at 10,000 rpm for 10 minutes to obtain serum. Next, a serum spike was prepared by mixing 50 µL of the resulting serum with 200 µL of an acetonitrile:water mobile phase (60:40, v/v), followed by vortexing until homogeneous. The mixture was then centrifuged again at 10,000 rpm for 10 minutes. It was then filtered using a 0.22 µm PTFE membrane before being injected into the HPLC system (Anukunwithaya et al., 2017).

Data Analysis

Data processing was performed by converting the HPLC peak area to asiaticoside concentration using the standard calibration curve equation. The resulting plasma concentration-time data were used to calculate pharmacokinetic

parameters (Cmax, Tmax, and A AUC_{0}–t) using Microsoft Excel. The area under the plasma concentration-time curve (AUC_{0}–t) from 0 to 360 minutes was calculated using the linear trapezoidal method. Sample concentrations below the lower limit of quantification (LLOQ) were treated as zero for pharmacokinetic analysis. Relative bioavailability was subsequently calculated by comparing the AUC_{0}–t values of the SNEDDS group to those of the non-SNEDDS extract group, as the 360-minute sampling framework adequately captured the primary absorption and elimination phases of asiaticoside. Differences in pharmacokinetic parameters between the two groups were statistically analyzed using IBM SPSS Statistics. Normality was tested using the Shapiro–Wilk test, and homogeneity was assessed using Levene's test, with a significance level of $p > 0.05$. The normally distributed and homogeneous data were analyzed using an independent samples t-test, while data that were not normally distributed were analyzed using the Mann–Whitney test with a significance level of $p < 0.05$ (Xing et al., 2017).

RESULTS AND DISCUSSION

Ethanolic Extract from *Centella Asiatica* Leaves

As reported in a previous study (Melati & Setiyadi, 2025), the extraction process of 250 g of dried powder (simplisia) via maceration using 96% ethanol yielded 82 g of a dense dark-green viscous extract, corresponding to a yield of 32.8%.

Self-Nanoemulsifying Drug Delivery Systems

The soybean oil, Tween 20, and PEG 400-based Self-Nanoemulsifying Drug Delivery System (SNEDDS) formulation (5:4:1 ratio) for *Centella asiatica* leaf extract reported by (Melati & Setiyadi, 2025) exhibited optimal nanoemulsion characteristics. The formulation presented a mean droplet size of 127.6 ± 17.78 nm, a polydispersity index (PDI) of 0.33 ± 0.05 (monodisperse), and a zeta potential of −45.86 ± 0.94 mV. Spontaneous nanoemulsification efficiency was demonstrated by a rapid emulsification time (<1 min) across various media: 47.33 s in distilled water, 52.66 s in simulated gastric fluid, and 40.33 s in simulated intestinal fluid, with percent transmittance values exceeding 85%, confirming high clarity and nanometer-scale droplet range. Furthermore, the system remained stable without phase separation or coalescence during dilution stability testing at 37°C for 4 h, ensuring that the SNEDDS characteristics were consistently maintained throughout the gastrointestinal tract.

Analysis of Asiaticoside Content in Extract and SNEDDS

The determination of asiaticoside content in the extract and SNEDDS was performed using the HPLC-UV method. The asiaticoside concentration was calculated based on the calibration curve regression equation derived from standard solutions. The standard curve demonstrated a linear relationship between concentration and peak area, with the regression equation $y = 167827x + 33084$ and an R^2 value of 0.996. Based on the analysis results, the asiaticoside content in the extract was 0.0266 ± 0.0013 mg per 100 mg of extract (n = 3). Meanwhile, in the SNEDDS formulation used 1 mL, the asiaticoside concentration was equivalent to 4.033 ± 0.313 µg/mL. These assay results subsequently served as the basis for determining the volume of asiaticoside administered to the test animals.

Method Validation

Prior to its use for the analysis of biological samples, the HPLC method was validated in accordance with guidelines, covering selectivity, linearity, sensitivity, accuracy, and precision. The validation results for the asiaticoside analysis method in rat serum are presented in Tabel 1.

Table 1. HPLC method validation results

Validation Parameter	Result
Selectivity	Matrix interference: 16.92–17.03%
Linearity	y=107004x+4935,2; R^2 = 0.9995
Sensitivity	%Recovery = 94.48 %; %CV = 1,163%
Accuracy	LLOQ: 93.43%; LQC: 103.66%; MQC: 102.91%; HQC: 102.98%
Precision	LLOQ: 0.103%; LQC: 0.044%; MQC: 0.111%; HQC: 0.110%

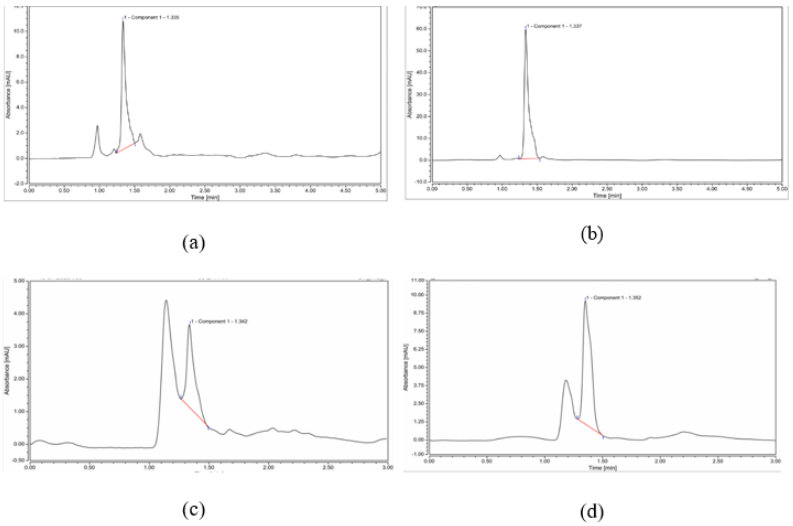


Figure 1. HPLC chromatogram of (a) blank serum (b) asiaticoside LLOQ (c) extract sample (d) SNEDDS sample

The validation results demonstrated that the HPLC method used met the acceptance criteria for the analysis of asiaticoside in rat serum. For Selectivity testing demonstrated that the percentage of interference in blank serum ranged from 16.92% to 17.03%. All values fell below the maximum limit mandated by (Food and Drug Administration, 2018), which is less than 20% of the mean response of the LLOQ. This indicates that endogenous components in the serum matrix do not exert significant interference on the detection of asiaticoside at the retention time of the analyte.

Based on linear regression analysis, the equation obtained was y = 107004x + 4935.2 with a coefficient of determination (R^2) of 0.9995. A coefficient of determination close to 1 establishes a linear relationship between asiaticoside concentration and peak area, demonstrating that an increase in asiaticoside concentration produces a proportionally higher detector response.

In sensitivity testing, a concentration of 0.39 µg/mL met the accuracy and precision criteria according to (Food and Drug Administration, 2018), with percent recovery falling within 80%–120% and percent Coefficient of Variation (%CV) not exceeding 20%. Consequently, this concentration was established as the Lower Limit of Quantification (LLOQ).

Regarding accuracy, the mean recovery values for LLOQ, LQC, MQC, and HQC were 93.43%, 103.66%, 102.91%, and 102.98%, respectively. All recovery values satisfied the acceptance range required by (Food and Drug Administration, 2018), namely 80%–120% for LLOQ and 85%–115% for QC samples. These results

demonstrate that the developed HPLC method possesses good accuracy, providing asiaticoside concentration measurements close to the nominal values.

For precision, the %CV values for LLOQ, LQC, MQC, and HQC were 0.103%, 0.044%, 0.111%, and 0.110%, respectively. All %CV values were well below the acceptance limits set by (Food and Drug Administration, 2018), which are less than 20% for LLOQ and less than 15% for QC samples. The low %CV values indicate minimal variation between repeated measurements, demonstrating excellent repeatability.

Serum sample stability during analysis was well preserved using freeze thaw cycles, demonstrating that asiaticoside did not undergo significant degradation. To prevent analyte breakdown at room temperature, serum preparation was always performed on a freshly prepared basis, and samples were injected into the HPLC system immediately. This analyte stability and minimal matrix interference were further corroborated by the excellent accuracy (% recovery of 93.43%–103.66%) and precision (%CV of 0.044%–0.111%). Overall, the analytical method meets the precision parameters for quantifying asiaticoside concentrations in rat serum.

Bioavailability Testing

Bioavailability testing was conducted to compare the pharmacokinetic profiles of asiaticoside following the oral administration of *C. asiatica* leaves ethanolic extract and SNEDDS forms to wistar rats (*Rattus norvegicus*). Serum asiaticoside levels were monitored at various sampling time points to observe changes in drug concentration throughout the observation period. The comparison between the two groups was subsequently used to determine the effect of the SNEDDS formulation on the absorption and systemic exposure of asiaticoside (Tawinwung et al., 2021).

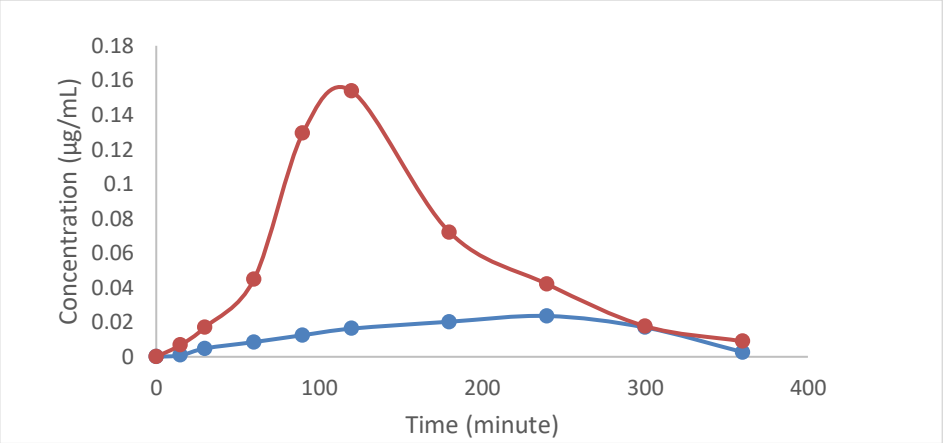


Figure 2. Asiaticoside concentration-time profiles after oral administration of ethanolic ● extract and ● SNEDDS. Data are presented as mean ± SD (n=5)

The serum asiaticoside concentration profile shown in figure 2 illustrates the differences between the c. asiatica leaves ethanolic extract group and the SNEDDS formulation group following oral administration. Samples were collected at 0, 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes post-administration. In the extract group, asiaticoside concentration increased gradually, reaching a peak of 0.02362 ± 0.00207 µg/mL at 240 minutes; conversely, in the SNEDDS group, the concentration rose more rapidly, reaching a peak of 0.15374 ± 0.00956 µg/mL at 120 minutes. The rate of increase in asiaticoside concentration was slower in the extract group compared to the SNEDDS group. This pattern is likely attributed to the limited aqueous solubility of asiaticoside, which can restrict the compound's availability for oral absorption (Annisa et al., 2023). This finding aligns with research indicating that asiaticoside within *C. asiatica* extract exhibits low oral bioavailability specifically, less than 1% (Anukunwithaya et al., 2017). The SNEDDS group demonstrated a faster rise in asiaticoside concentration and achieved a higher Cmax than the extract group. These results indicate that the SNEDDS formulation enhances asiaticoside availability for oral absorption. SNEDDS is a delivery system composed of oil, surfactants, and co-surfactants designed to improve the solubility and absorption of compounds with poor water solubility, thereby offering the potential to enhance oral bioavailability (Sabillah et al., 2025).

Data Analysis

The data was performed to determine pharmacokinetic parameters, including Cmax, Tmax, $AUC_{0–t}$, and relative bioavailability. These parameters were used to evaluate the effect of the SNEDDS formulation on the absorption and bioavailability of asiaticoside compared to

the ethanolic extract of *C. asiatica.*

Table 2. Pharmacokinetic parameter (mean ± SD (n=5))

Parameter	Extract	SNEDDS
Cmax (µg/mL)	0,0236214 ± 0,002067021	0,15374 ± 0,009560082
Tmax (min)	240 ± 0	120 ± 0
AUC_{0}–t (µg·min/mL)	5,22352 ± 0,16073711	20,8248 ± 0,52093414
Relative bioavailability (%)	100	398,68

Based on the analysis of pharmacokinetic parameters in Table 2, the SNEDDS formulation demonstrated increased bioavailability of asiaticoside compared to the ethanolic extract of *C. asiatica*. This is evidenced by the higher Cmax value for the SNEDDS group (0.15374 ± 0.00956 µg/mL) compared to the extract group (0.02362 ± 0.00207 µg/mL), indicating a greater amount of asiaticoside absorbed into the systemic circulation (Wardiyah et al., 2026). Furthermore, the Tmax value for the SNEDDS group (120 minutes) was shorter than that of the extract group (240 minutes), indicating that the SNEDDS formulation accelerated the absorption process of asiaticoside following oral administration (Tawinwung et al., 2021). The AUC_{0}–t value for the SNEDDS group (20.8248 ± 0.5209 µg·min/mL) was also higher than that of the extract group (5.2235 ± 0.1607 µg·min/mL), reflecting increased total exposure to asiaticoside in the systemic circulation. Relative bioavailability calculations revealed that the SNEDDS formulation exhibited a bioavailability of 398.68%—approximately 3.99 times higher than that of the ethanolic extract of *C. asiatica* (Lin et al., 2023). This enhancement is attributed to the ability of SNEDDS to spontaneously form nanoemulsions with small droplet sizes, thereby increasing solubility, expanding the contact surface area, and enhancing asiaticoside absorption in the gastrointestinal tract. Thus, the SNEDDS formulation has been shown to improve the bioavailability of asiaticoside compared to the administration of the unformulated ethanolic extract of *C. asiatica* leaves (Annisa et al., 2023).

CONCLUSION

The results demonstrated that the

SNEDDS formulation tended to improve the oral absorption and systemic bioavailability of asiaticoside compared with the conventional *C. asiatica* ethanolic extract. Pharmacokinetic evaluation revealed that SNEDDS potentially increased the Cmax from 0.02362 ± 0.00207 to 0.15374 ± 0.00956 µg/mL, shortened the Tmax from 240 to 120 minutes, and elevated the $AUC_{0–t}$ from 5.22352 ± 0.16074 to 20.8248 ± 0.52093 µg·min/mL. Nevertheless, the detected serum concentrations fell below the validated Lower Limit of Quantification (LLOQ of 0.39 µg/mL). Thus, the relative bioavailability of asiaticoside from the SNEDDS formulation reached 398.68%, representing an approximately 3.99 fold increase compared with the ethanolic extract. These findings indicate that SNEDDS has the potential to enhance both the rate and extent of asiaticoside absorption, serving as a promising delivery system for poorly water soluble bioactive compounds.

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