Trop J Nat Prod Res, March 2025; 9(3): 960 - 966 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Evaluation of Herbonanoceutical Formulations of Tamoenju (*Hibiscus surattensis* L.) Leaf Extract

Yuliet[1] Susanto 1*, Khildah Khaerati[1], Nela Sharon[1], Joni Tandi[2]

[1]*Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu-94118, Central Sulawesi, Indonesia*
[2]*Department of Pharmacy, College of Pharmaceutical Sciences Pelita Mas, Palu-94111, Indonesia*

ARTICLE INFO

Article history:
Received 11 February 2024
Revised 01 September 2024
Accepted 01 March 2025
Published online 01 April 2025

ABSTRACT

Tamoenju (*Hibiscus surattensis* L.) is a natural ingredient with antioxidant activity and potential applications in complementary therapy for diabetes mellitus and associated complications. Herbonanoceutical preparations in the form of self-nano-emulsifying drug delivery system (SNEDDS) are well-known for increasing solubility and optimizing pharmacological activity. Therefore, this study is aimed at evaluating the herbonanoceutical formulations of tamoenju leaf extract for its physicochemical characteristics, and stability. Tamoenju leaves were extracted by maceration in 96% ethanol. The extract was subjected to solubility test in oil, surfactant, and cosurfactant. Herbonanoceutical formulations of tamoenju leaf extract (SNASET) were prepared using eight different formulae (F1 – F8) containing different concentrations of oils, surfactants, and cosurfactants. Oil:surfactant+cosurfactant ratio 1:9 was used in all the formulae. The formulations were analysed for their physicochemical characteristics and stability. The parameters evaluated include; transmittance, dispersibility, particle size, zeta potential, polydispersity index, pH, viscosity, robustness, and thermodynamic stability. The results showed that three of the formulae; F1 (tween 80:propylene glycol, 2:1), F2 (tween 80:propylene glycol, 3:1), and F7 (Cremophor RH:PEG 400, 1:1) produced the most optimal formulations based on the physicochemical characteristics and stability. F1, F2, and F7 had the following values for particle size (32.22 nm, 8.49 nm, 19.33 nm), transmittance (99.95-99.98%), polydispersibility index (0.1550, 0.1051, 0.1630), pH (5.11, 7.97, 5.46), viscosity (372 cP, 440 cP, 1116 cP), respectively. The three formulations met grade A criteria for dispersibility, and were thermodynamically stable. The study has successfully formulated tamoenju leaves extract into herbonanoceutical formulations that may find potential application as natural health products.

Keywords: Characterization, Formulation, *Hibiscus surattensis* L., Optimation, Stability.

Introduction

Tamoenju (*Hibiscus surattensis* L.) leaves extract is a local wisdom in Central Sulawesi with significant antidiabetic potential.[1,2] Several studies have shown that the plant contains alkaloids, flavonoids, tannins, quinones, and triterpenoids, which are responsible for the bioactivities of the plant. Compounds identified in tamoenju include 6-hydroxy kaempferol-3-*O*-glucoside, buddlenoid A, kaempferol, morin, and trifolin.[3] Each of these compounds has been found to possess antioxidant and antidiabetic effects.[4,5] Despite its therapeutic potentials, direct oral administration of tamoenju leaf extract is not considered acceptable by the local community due to its discomforting effect on the gastrointestinal tract, which suggest the need for a more acceptable method of administration. Tamoenju leaf extract has low solubility and poor bioavailability due to the aglycone component of its flavonoid compounds. To address these challenges, several studies have recommended the use of drug delivery systems, such as self-nanoemulsifying drug delivery system (SNEDDS).

*Corresponding author. Email: yuliet_susanto@yahoo.com
Tel: +62-821-9521-3868

Citation: Susanto Y, Khaerati K, Sharon N, Tandi J. Evaluation of Herbonanoceutical Formulations of Tamoenju (*Hibiscus surattensis* L.) Leaf Extract. Trop J Nat Prod Res. 2025; 9(3): https://doi.org/10.26538/tjnpr/v9i3.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

In this context, the system has the ability to enhance patient compliance during drug administration and mask the unpleasant taste associated with the use of lipid carrier formulae.[6-9] According to previous studies, SNEDDS is composed of an isotropic mixture of oil, surfactant, cosurfactant, and drug, which spontaneously forms nanoemulsions shortly after mixing with water. The advantage of this preparation include the ability to form nanoemulsions spontaneously in the gastrointestinal tract, with the size of the droplets produced being in the nanometer range.[10,11] SNEDDS represents a modern dosage form that can increase the solubility and bioavailability of drugs. The system is expected to provide the same pharmacological effect as non-SNEDDS preparations, even at lower doses. The main components of SNEDDS include; 1) oil as a drug carrier, 2) surfactant responsible for emulsifying the oil into water and maintaining interfacial stability, and 3) cosurfactant for improving drug incorporation or facilitating nanoemulsification.[12] The oil component aids in dissolving water-insoluble drugs, enhances dissolution in the intestine, and promotes entry into the lymphatic system. Drug transport through this system bypasses first-pass metabolism, thereby increasing bioavailability. SNEDDS can also improve the physical and chemical stability of preparations on long-term storage.[13] The characteristics of the final products obtained are affected by various factors, including oil-surfactant ratio, polarity, emulsion droplet charge, oil phase type and concentration, cosurfactant, pH, emulsification temperature, and physicochemical properties.[14]

Arising from previous research findings, tamoenju leaves have various medicinal properties that hold promise for its development into useful health products. Despite its potentials, there is limited information on the application of tamoenju leaves in herbonanoceutical formulations. Therefore, this study aim to evaluate herbonanoceutical preparations of tamoenju leaves extract in the form of SNEDDS by assessing its physicochemical characteristics, and stability. The primary objective is

to obtain optimal formulae with the best ratio of oil, surfactant, and cosurfactant, for self-nanoemulsifying formulation with good physicochemical characteristics and stability. The findings from the study are expected to serve as a foundation for the development of tamoenju leaves into a more effective and safer herbonanoceutical formulation, and this will make a significant contribution to the field of herbal medicine through the use of nanotechnology.

Materials and Methods

Chemicals and equipment
The chemicals used include olive oil, virgin coconut oil (VCO), propylene glycol, Tween 20, Tween 80, and polyethylene glycol (PEG) 400 (Brataco, Indonesia). Others included Cremophor RH 40 (BASF, Indonesia), ethanol 96% pa (Merck), distilled water (Waterone), HCl, and phosphate buffer pH 8 (Sigma Aldrich). The following instruments were used; electronic balance (OHAUS Analytical Balance EX423G), pH meter (Horiba LAQUA PH1100-S), Brookfield Ametek viscometer, PSA (particle size analyzer) (Horiba Scientific, nanoparticle analyzer SZ-100), magnetic stirrer, UV-Vis spectrophotometer (Cecil CE7410), rotary evaporator (Buchi R-300), waterbath (Memmert WNB 14), sonicator (Qsonica), blender (Miyako), oven (Memmert Oven), and zeta sizer (Horiba Scientific SZ-100).

Plant collection and identification
Tamoenju leaves were collected from Alindau village, Sindue Tobata District, Donggala Regency, Central Sulawesi Province (GPS coordinates: -0.33586, 119.77302) in January 2023. The plant material was identified by a taxonomist (Mr. Moh. Iqbal) at the Plant Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, Central Sulawesi, Indonesia. Herbarium specimen with voucher number 031/UN28.1.28/BIO/2023 was deposited.

Plant extraction
A total of 1750 g of dried tamoenju leaf powder was macerated in 96% ethanol at a 1:5 ratio of plant material:solvent for five days, with daily stirring for about 15 min. The resulting extract was concentrated using a rotary evaporator, followed by drying on a water bath at 50ºC.

Solubility test of tamoenju leaf extract
Tamoenju leaf ethanol extract (100 mg) was dissolved separately in 10 mL each of olive oil, VCO, tween 80, tween 20, Cremophor RH, PEG 400, and propylene glycol. The mixture was heated on a water bath at 40°C for 10 min. To facilitate the dissolution process, the mixture was sonicated for 15 min.[15] The resulting formulation of tamoenju leaf extract was regarded as SNASET.

Optimization of tamoenju leaf extract herbonanoceutical formulae (SNASET)
Mixtures of the oil, surfactant, and cosurfactant at different ratios were made up to a final volume of 5 mL in a vial. Each of the mixtures was homogenized using a magnetic stirrer for 30 min, then sonicated for 16 min, followed by heating on a water bath at 45°C for 10 min. The mixture was allowed to settle for 24 h at room temperature to examine its homogeneity.[16] The most optimal formulae (clear and homogeneous) were selected to be reformulated with 100 mg tamoenju leaf extract.

Preparation of SNASET herbonanoceutical
Approximately 100 mg tamoenju leaf extract was added to 100 mL oil, surfactant, and cosurfactant mixture, homogenized with a magnetic stirrer for 30 min, and then ultrasonicated at 40% amplitude for 20 min.

Physicochemical analysis of SNASET herbonanoceutical preparation
Determination of transmittance
A total of 100 µL herbonanoceutical formula was diluted to 100 mL using distilled water. The percentage transmittance was measured at a wavelength of 650 nm using a UV-Vis spectrophotometer. Distilled water was used as blank.[17]

Determination of particle size, polydispersity index, and zeta potential
The particle size, polydispersity index, and zeta potential were determined using a Particle Size Analyzer (PSA) (Beckman Coulter LS 13 320). A total of 100 µL SNASET formula was dissolved in 100 mL distilled water and subsequently analyzed with a PSA device.[18]

pH test
The pH of the preparation was determined using a pH meter, which was initially calibrated with standard buffer solutions of pH 4 and 7.[19]

Viscosity test
Viscosity measurements were done using a Brookfield viscometer. A total of 14 mL test sample preparation was poured into the cup and placed on the solvent trap, while the viscometer was set at 100 rpm.[20]

Dispersibility test
The dispersibility test was conducted using a type II dissolution test kit. The formula (1 mL) was added into 500 mL distilled water in a dissolution flask and stirred at 50 rpm at a temperature of 37 ± 0.5ºC. Visual observations were performed according to the degree of emulsification. The level of dispersibility was determined according to the guidelines presented in Table 1. For acceptability, the preparation must meet the criteria for grade A or B.[21]

Table 1: Dispersibility test grade

Grade	Description
A	The system formed nanoemulsion quickly (<1 min) with a clear appearance
B	The system formed nanoemulsion rapidly (<1 min) with a dull appearance
C	Milky white emulsion formed in < 2 minutes
D	Non-clear grayish-white emulsion with a slightly oily appearance and slow-to-form nanoemulsion (> 2 min)
E	Slightly emulsified and visible large oil globules on the surface

Test for robustness
Approximately 1 mL of each SNASET formula was mixed with 100 mL distilled water, 0.1 N HCl, and phosphate buffer pH 6.8. The mixture was stirred with a magnetic stirrer at 100 rpm at 37°C. The solution was stored at room temperature for 24 h and visually observed for any signs of phase separation.[22]

Thermodynamic stability test
The thermodynamic stability test was conducted in three stages, namely; centrifugation, heating-cooling, and freeze-thaw cycle. The centrifugation test was carried out at 4000 rpm for 30 min. All the formulae were visually observed for the presence or absence of phase separation and precipitation. Subsequently, qualified SNASET preparations were subjected to heating-cooling and freeze-thaw cycle tests. The heating-cooling test was performed for 3 cycles between refrigerator temperatures of 4ºC and 45ºC with not less than 48 h at each storage temperature. The selected formulae were observed for the presence or absence of cracking, creaming, phase separation, coalescence, or phase inversion. The freeze-thaw cycle test was performed by storing the preparation at -21ºC and 25ºC with not less than 48 h storage at each temperature for 3 cycles. The preparation was observed for the presence or absence of phase separation or precipitation.[23]

Statistical analysis
All experiments were done in triplicates, and data were presented as mean ± standard deviation (SD).

Results and Discussion

Extract yield

The extraction of tamoenju leaves by maceration in ethanol at room temperature yielded 382.94 g of dry ethanol extract, corresponding to a percentage yield of 21.88%.

Optimization outcome of tamoenju leaf extract herbonanoceutical formulae (SNASET)

Solubility test was conducted to determine the proportion of oil, surfactant, and cosurfactant that can optimally dissolve tamoenju leaf extract. The selection of a suitable carrier in the formulae was important to prevent drug precipitation in the intestinal lumen.[24,25] Surfactant and cosurfactant in nanoemulsion systems work in synergy to form a good and flexible interfacial system, which reduces the surface tension to near zero, thereby supporting the formation of stable nano-sized globules.[26] According to the optimization results as presented in Table 2, the composition of surfactant:cosurfactant; Tween 80:propylene glycol, and tween 20:propylene glycol at ratios 1:1, 2:1, and 3:1, and surfactant:cosurfactant; Cremophor RH:PEG 400 at ratios 1:1 and 3:1, exhibited good mixing with VCO compared to olive oil. A more balanced surfactant-cosurfactant composition ratio of 1:1 and 2:1, tended to produce a more homogeneous mixture compared to an unbalanced ratio of 3:1, 2:3, and 1:2. This indicated the importance of maintaining a balance between surfactants and cosurfactants to achieve good emulsion stability. The effective combination of surfactants and cosurfactants could vary depending on the type of oil used.[27,28]

Table 2: Virgin coconut oil (VCO) oil and olive oil with surfactant and cosurfactant formulae

Surfactant: cosurfactant composition ratio	**VCO**			**Olive oil**		
	Results of oil: surfactant- cosurfactant composition ratio (1:9)					
	T80:PG	**T20:PG**	**Cremophor RH: PEG 400**	**T80:PG**	**T20:PG**	**Cremophor RH: PEG 400**
1:1	✓	✓	✓	x	x	x
2:1	✓	✓	x	x	x	x
3:1	✓	✓	✓	x	x	x
3:2	x	x	x	x	x	x
2:3	x	x	x	x	x	x
1:3	x	x	x	x	x	x
1:2	x	x	x	x	x	x

✓ = homogeneous
x = separation (within 24 hours)
VCO = Virgin coconut oil, T80 = Tween 80, T20 = Tween 20, PG = Propylene glycol, PEG = Polyethylene glycol.

Physicochemical characteristics of SNASET herbonanoceutical preparation

The study optimized eight herbonanoceutical formulae (Table 3), which were tested for their physicochemical characteristics and stability. Surfactants were used to reduce the surface tension between two immiscible phases such as oil and water, helping in the formation of emulsions, while, cosurfactants were used to increase the stability of the resulting emulsion by complementing the effect of surfactants in reducing surface tension.

Table 3: Herbonanoceutical formulae of tamoenju leaf extract (SNASET)

Formula	**Extract**	**VCO: Surfactant+ cosurfactant**	**Surfactant (Tween 80)**	**Surfactant (Tween 20)**	**Surfactant (Cremophor RH)**	**Cosurfactant (Propyleneglycol)**	**Cosurfactant (PEG 400)**
F1	100 mg	1:9	2	-	-	1	-
F2	100 mg	1:9	3	-	-	1	-
F3	100 mg	1:9	3	-	-	2	-
F4	100 mg	1:9	-	2	-	1	-
F5	100 mg	1:9	-	3	-	1	-
F6	100 mg	1:9	-	3	-	2	-
F7	100 mg	1:9	-	-	1	-	1
F8	100 mg	1:9	-	-	3	-	1

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

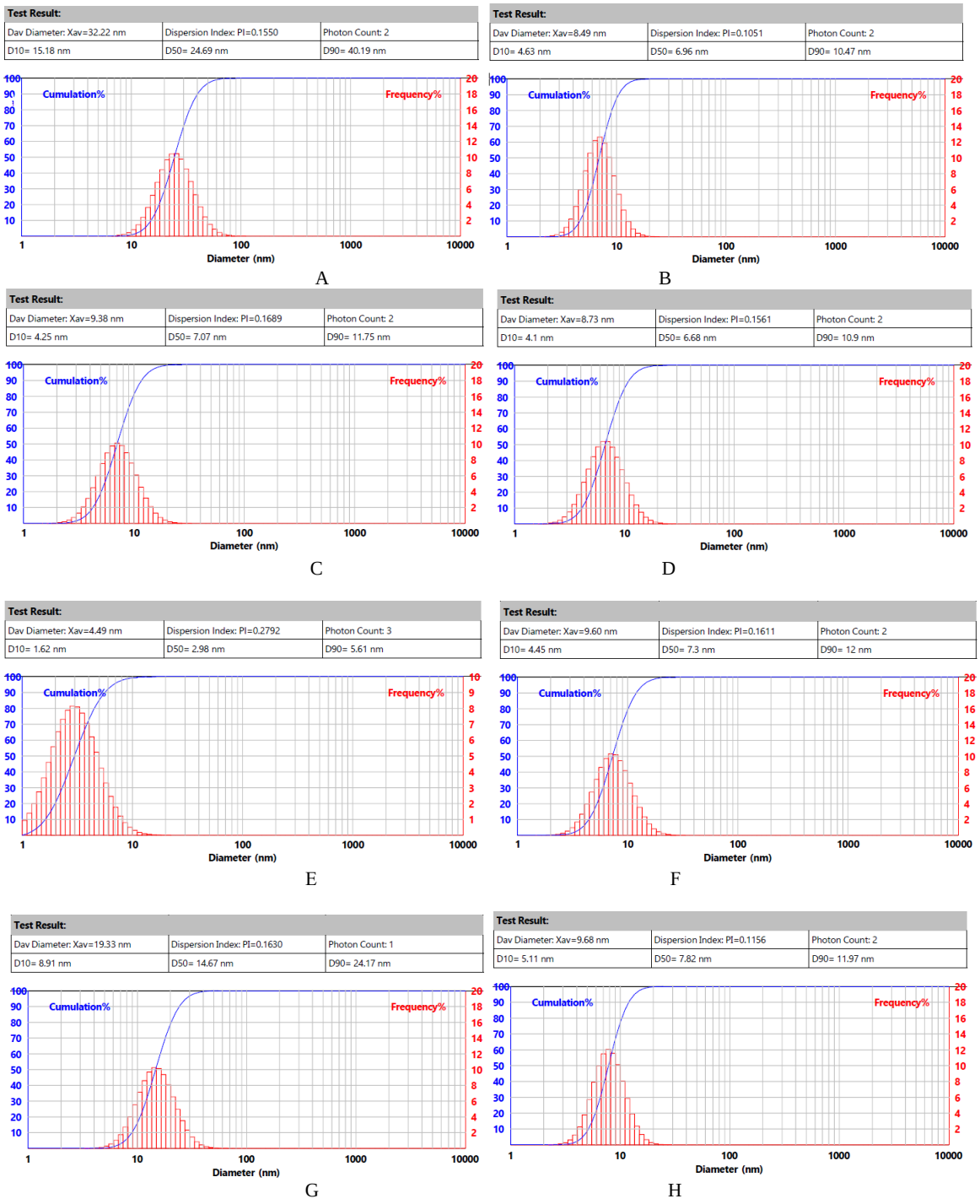

Figure 1: Particle size distribution of herbonanoceutical formulation of tamoenju leaf extract (SNASET) **(A)** Formula F1; **(B)** Formula F2; **(C)** Formula F3; **(D)** Formula F4; **(E)** Formula F5; **(F)** Formula F6; **(G)** Formula F7 **(H)** Formula F8

The percentage transmittance, particle size, polydispersibility index, zeta potential, pH, and viscosity of the preparation are presented in Table 4. All the formulae had a transmittance value of more than 95%. This showed that the formulae could produce a perfectly dispersed emulsion with particle size in the nanometer range (<100 nm), with a transparent and clear appearance. Consequently, it can be inferred that the emulsion droplets formed exhibited the potential to form a nanoemulsion. High-quality nanoemulsion formulae should have a clear appearance with a transmittance range of 90 to 100%.[29,30] In this study, the particle size of the nanoemulsion formed ranged from 4.49 to 32.22 nm. The particle size distribution of the herbonanoceutical formulation of tamoenju leaf extract can be seen in Figure 1. An emulsion system with a droplet size ranging between 20 - 500 nm belong to the nanoemulsion category.[31] A small globule size could increase the surface area, leading to faster absorption, and increased bioavailability.[32]

The polydispersity index of a nanoemulsion system describes the homogeneity and size distribution of the globules.[33] The polydispersity index of SNASET obtained was less than 0.5 on the average, which indicated a homogeneous size distribution. Zeta potential served as an indicator of herbonanoceutical stability because differences in the charge between particles affect the repulsive force. A preparation is considered stable when the zeta potential exceeds ±25 mV.

Table 4: Physicochemical characteristics of herbonanoceutical formulations of tamoenju leaf extract (SNASET)

Formula	Transmittance (%)	Particle Size (nm)	Polydispersity Index	Average pH ± SD	Average viscosity (cP) ± SD	Zeta potential
F1	99.95	32.22	0.1550	5.11 ± 0.01	372 ± 1	-30.15 ± 0.35
F2	99.98	8.49	0.1051	7.97 ± 0.01	440 ± 3	-27.25 ± 0.35
F3	99.93	9.38	0.1689	8.01 ± 0.12	306 ± 6	-32.20 ± 0.28
F4	99.98	8.73	0.1561	6.76 ± 0.01	190 ± 9	-28.50 ± 0.42
F5	99.31	4.49	0.2792	6.24 ± 0.01	235 ± 2	-23.95 ± 0.64
F6	99.20	9.60	0.1611	6.17 ± 0.05	208 ± 3	-25.60 ± 0.42
F7	99.98	19.33	0.1630	5.46 ± 0.01	1116 ± 6	-24.05 ± 0.64
F8	99.93	9.68	0.1156	7.06 ± 0.02	1758 ± 6	-29.2 ± 0.57

High zeta potential of more than 25 mV or less than -25 mV indicate a stable preparation that could resist the agglomeration of particles caused by repulsive forces.[34] The zeta potential test results showed that formulae F1, F2, F3, F4, F6, and F8 met the requirements, while F5 and F7 did not.

Based on the test results presented in Table 4, all SNASET herbonanoceutical formulae had normal pH levels for oral use.[35] The pH measurement was aimed at determining whether the pH of the system is within the acceptable range for oral administration. In this study, the viscosity of the SNASET preparations was in the range of 190-1758 cP, although, nanoemulsion preparations should not be viscous. High viscosity could inhibit drug release from the preparation. Highly viscous preparation results in the entrapment of active substance in the carrier, inhibiting it release, and ultimately decreasing the desired therapeutic effect.

The dispersibility test was conducted to determine the process and time required for nanoemulsion formation *in-vitro* by visually observing some set criteria in a grading system (Table 1).[36] All formulae met the grade A criteria for the dispersibility test, indicating that SNASET preparation formed a clear, transparent nanoemulsion system, without phase separation. This implies that the SNEDDS is suitable for oral administration. While in the GIT, the nanoemulsion system presents as an emulsified globules without any phase separation. The absence of phase separation or turbidity is a positive indicator, suggesting that the nanoemulsion is stable, and the oil droplets are effectively covered by surfactant and cosurfactant. Based on the emulsification time, visual appearance, and transmittance of the nanoemulsion, formulae F1, F2, F7, and F8 were categorized as grade A (Table 5).

Table 5: Dispersibility, robustness, and thermodynamic stability of SNASET

Formula	Evaluation								
	Dispersibility			Robustness			Thermodynamics		
	Emulsification time	Appearance	Grade	Aquadest	HCl 0.1 N	Phosphate Buffer pH 6.8	Centrifugation	Heating cooling	Freeze-thaw
F1	5.29”	Clear	A	Clear, stable	Clear, stable	Clear, stable	Stable	Stable	Stable
F2	30.91”	Clear	A	Clear, stable	Clear, stable	Clear, stable	Stable	Stable	Stable
F3	1‘ 4“	Milky white	C	Milky white, stable	Milky white, stable	Milky white, stable	Stable	Stable	Stable
F4	1’ 6”	Grayish white	C	Separation	Separation	Separation	Separation	Separation	Separation
F5	1’ 37”	Grayish white	C	Separation	Separation	Separation	Separation	Separation	Separation
F6	1’ 17”	Milky white	C	Separation	Separation	Separation	Separation	Separation	Separation
F7	28.45”	Clear	A	Clear, stable	Clear, stable	Clear, stable	Stable	Stable	Stable
F8	37”	Clear	A	Clear, stable	Clear, stable	Clear, stable	Stable	Stable	Stable

The emulsification time was measured to estimate the efficiency of spontaneous emulsification. SNEDDS must be completely dispersed rapidly when mixed in aqueous media with minimal stirring.

The robustness test was conducted to determine the ability of SNASET to form a stable system at the different pH values within the gastrointestinal tract. Formulae F1, F2, F7, and F8 were effectively dispersed in distilled water, 0.1 N HCl (gastric condition), and phosphate-buffered saline pH 6.8 (small intestinal condition). The indicate that formulae F1, F2, F7, and F8 would be stable in acidic and alkaline pH as well as in the presence of electrolytes in the gastrointestinal tract. This stability may be attributed to the surfactant and cosurfactant used.[37,38]

Thermodynamic stability of SNASET herbonanoceutical preparation

The thermodynamic test was primarily aimed at determining the stability of SNASET preparations. The results indicated that F1, F2, F3, F7, and F8 had good thermodynamic stability. A centrifugation test was conducted to determine the resistance of the preparations to gravity. The small globule size of the preparations minimized the impact of gravitational force and Brownian motion on the particles, preventing creaming and sedimentation.[39] This test was also conducted to determine the effect of shock on the physical appearance during product transportation. Other tests, such as the heating, cooling and freeze-thaw tests, were carried out to assess the resistance of SNASET to separation and precipitation due to temperature changes.[40] The results showed no

phase separation under extreme storage conditions for F1, F2, F3, F7, and F8. Formulae F1, F2, and F8 showed a clear appearance and varying, but satisfactory emulsification time. Based on the thermodynamic tests (centrifugation, heating/cooling, and freeze/thaw) F1, F2, and F8 maintained good stability, whereas, on the basis of the physicochemical characteristics and stability evaluation, three SNASET formulae (F1, F2, and F7) displayed good physicochemical characteristics and physical stability.

Conclusion

The findings from the study showed that VCO oil carrier, tween 20, tween 80, Cremophor RH, propylene glycol, and PEG 400 affected the physicochemical features and stability of SNASET herbonanoceutical preparations. Tamoenju leaves extract was made into SNASET herbonanoceutical preparations using eight different formulae. Three (3) of the formulae (F1, F2, and F7 met the requirements for the various physicochemical characteristics including percentage transmittance, particle size, polydispersity index, zeta potential, pH, viscosity, dispersibility, and robustness. In addition, homogeneous and stable SNASET formulations were produced, characterized by the absence of phase separation and precipitation. This study could significantly contribute to the advancement of herbonanoceutical science and its practical applications. These formulations could be commercialized, providing a new class of natural health products that combined the benefits of traditional herbal medicine with modern nanotechnology.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them**.**

Acknowledgments

This study was supported by a Regular Fundamental Research Grant from the Indonesia Ministry of Education, Culture, Research, and Technology in 2023, with contract number 1460/UN28.2/PL/2023.

References

1. Yuliet, Sukandar EY, Atik N, Adnyana IK. Insulin secretion and repairing pancreatic tissue damage on diabetic mice treated with the extract and active fraction of *Hibiscus surattensis* L. Leaves. J Pharm Pharmacogn Res. 2021; 9(4):454-464.
2. Susanto Y, Sukandar EY, Adnyana IK, Tandi J. Efficacy of the *Hibiscus surattensis* L. leaves active fraction in reducing the levels of HbA1c, AGEs, and glucose optake in muscle cells of diabetic type 2 model rat. Indones J Pharm. 2022; 33(4):630-640.
3. Yuliet, Sukandar EY, Adnyana IK. Active subfractions, phytochemical constituents, dipeptidyl peptidase-IV inhibitory activity and antioxidant of leaf extract from H*ibiscus surattensis* L. Nat Prod J. 2020; 10(4):400-410.
4. Shehadeh MB, Suaifan GARY, Abu-Odeh AM. Plants secondary metabolites as blood glucose-lowering molecules. Molecules. 2021; 26(14):4333.
5. Sukhikh S, Babich O, Prosekov A, Kalashnikova O, Noskova S, Bakhtiyarova A, Krol, O, Tsvetkova E, Ivanova S. Antidiabetic properties of plant secondary metabolites. Metabolites. 2023; 13(4):513.
6. Nikam P, Jain A, Solanki D, Aher S. Revolutionizing pharmaceuticals: a deep dive into self nano emulsifying drug delivery systems. Int J Curr Pharm Res. 2024;16(1):1-9.
7. Rusminingsih E, Susanto H, Afifah DN, Martien R, Subagyo HW. Effectiveness of *Moringa oleifera* nanoparticles (self nano emulsifying drug delivery system) on insulin resistance in the prediabetes *Rattus norvegicus* model. Trop J Nat Prod Res. 2023; 7(11):5059-5066.
8. Puspita OP and Nugroho AK. Optimization of self-nanoemulsifying drug delivery system for Pterostilbene. J Food Pharm Sci. 2016; 4:18-24.
9. Erliyana M, Widyaningsih W, Wumu DA, Wulansari WF. Formulation of self-nano emulsifying drug delivery system (SNEDDS) red ginger extract (*Zingiber officinale var. rubrum*). Media Farm J Ilmu Farm. 2022; 19(2):133.
10. Nasr A, Gardouh A, Ghorab M. Novel solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of olmesartan medoxomil: Design, formulation, pharmacokinetic and bioavailability evaluation. Pharmaceutics. 2016; 8(3):20.
11. Sutoyo S, Amaria A, Sanjaya IGM, Hidayah R, Sari DP, Dwitarani N, Octavia FD, Fadzlillah NA. Synthesis of nanoherbal from ethanol extract of Indonesian fern *Selaginella plana* and antibacterial activity assay. Trop J Nat Prod Res. 2022; 6(1):44-49.
12. Buya AB, Beloqui A, Memvanga PB, Préat V. Self-nano-emulsifying drug-delivery systems: From the development to the current applications and challenges in oral drug delivery. Pharmaceutics. 2020; 12(12):1-52.
13. Aziz A, Zaman M, Khan MA, Jamshaid T, Butt MH, Hameed H, Rahman MS, Shoaib QA. Preparation and evaluation of a self-emulsifying drug delivery system for improving the solubility and permeability of ticagrelor. ACS Omega. 2024; 9(9):10522-10538.
14. Salawi A. Self-emulsifying drug delivery systems: a novel approach to deliver drugs. Drug Deliv 2022;29(1):1811-1823.
15. Purwanto URE, Sholikhah M, Munisih S. Formulation and physical characterization of essential oil Bangle (*Zingiber cassumunar* Roxb.) nanoemulsion gel. J Sci Technol Res Pharm. 2021; 1(1):1-11.
16. Hariyatno SP, Paramita V, Amalia R. The effect of surfactant, time and speed of stirring in the emulsification process of Soybean oil in water. J Vocat Stud Appl Res. 2021; 3(1):21-25.
17. Fitria A, Hanifah S, Chabib L, Uno AM, Munawwarah H, Atsil N, Pohara HA, Weuanggi DA, Syukri Y. Design and characterization of propolis extract loaded self-nano emulsifying drug delivery system as immunostimulant. Saudi Pharm J. 2021; 29(6):625-634.
18. Syukri Y, Martien R, Lukitaningsih E, Nugroho AE. Novel self-nano emulsifying drug delivery system (SNEDDS) of andrographolide isolated from *Andrographis paniculata* Nees: characterization, in-vitro and in-vivo assessment. J Drug Deliv Sci Technol. 2018; 47:514-520.
19. Yusuf VAJ, Soeratri W, Erawati T. The effect of surfactant combination on the characteristics, stability, irritability, and effectivity of Astaxanthin nanoemulsion as anti-ageing cosmetics. Trop J Nat Prod Res. 2023; 7(12):5509-5518.
20. Ahmad N, Ahmad R, Ahmad FJ, Ahmad W, Alam MA, Amir M, Ali A. Poloxamer-chitosan-based naringenin nanoformulation used in brain targeting for the treatment of cerebral ischemia. Saudi J Biol Sci. 2020; 27(1):500-517.
21. Prasanthi D, Meghana G, Navya V. Formulation and evaluation of solid-self nanoemulsifying drug delivery system of famotidine. Int J Pharm Sci Res. 2021; 12(11):5785-5799.
22. Winarti L, Suwaldi, Martien R, Hakim L. Formulation of self-nanoemulsifying drug delivery system of Bovine serum albumin using HLB (Hydrophilic-Lypophilic Balance) approach. Indones J Pharm. 2016; 27(3):117-127.

23. Abdelmonem R, Younis MK, Hassan DH, El-Gawad MA, Ahmed ES, Hassanein E, El-Batouty K, Elfaham A. Formulation and characterization of chlorhexidine HCl nanoemulsion as a promising antibacterial root canal irrigant: in-vitro and ex-vivo studies. Int J Nanomed. 2019; 14:4697-4708.
24. Kumar M, Bishnoi RS, Shukla AK, Jain CP. Techniques for formulation of nanoemulsion drug delivery system: A review. Prev Nutr Food Sci. 2019; 24(3):225-234.
25. Smail SS, Ghareeb MM, Omer HK, Al-Kinani AA, Alany RG. Studies on surfactants, cosurfactants, and oils for prospective use in formulation of ketorolac tromethamine ophthalmic nanoemulsions. Pharmaceutics. 2021; 13(4):467.
26. Rostinawati T. Review of surfactant use in curcumin drug delivery system. J Kartika Kim. 2022; 5(1):79-89.
27. Abdullah NA, Jufri M, Mun'im A, Saputri FC. Formulation and evaluation of two celastrol nanoemulsions prepared from two oils: Isopropyl myristate and virgin coconut oil. Int J Appl Pharm. 2022; 14(2):267-275.
28. Pudyastuti B and Wijaya TH. The effect of surfactant on the solubility of kencur rhizome ethanol extract in self-nanoemulsifying drug delivery system. Pharmaciana. 2022; 12(3):319.
29. Nurdianti L, Yuliana A, Firmansya A, Setiawan F. Formulation and characterization of buccal film nanoemulsion apigenin as antidiabetic. Indones J Pharm Sci Technol. 2022; 1(1):22.
30. Mohsin K, Alamri R, Ahmad A, Raish M, Alanazi FK, Hussain MD. Development of self-nanoemulsifying drug delivery systems for the enhancement of solubility and oral bioavailability of fenofibrate, A poorly water-soluble drug. Int J Nanomed. 2016; 11:2829-2838.
31. Gaber DA, Alsubaiyel AM, Alabdulrahim AK, Alharbi HZ, Aldubaikhy RM, Alharbi RS, Albishr WK, Mohamed HA. Nano-emulsion based gel for topical delivery of an anti-inflammatory drug: in vitro and in vivo evaluation. Drug Des Devel Ther. 2023; 17:1435-1451.
32. Anuar N, Sabri AH, Bustami Effendi TJ, Abdul Hamid K. Development and characterisation of ibuprofen-loaded nanoemulsion with enhanced oral bioavailability. Heliyon. 2020; 6(7):e04570.
33. Jusril NA, Abu Bakar SI, Khalil KA, Md Saad WM, Wen NK, Adenan MI. Development and optimization of nanoemulsion from ethanolic extract of *Centella asiatica* (NanoSECA) using D-optimal mixture design to improve blood-brain barrier permeability. Evid-Based Complement Alternat Med. 2022; 2022: 3483511.
34. Osonwa UE, Okechukwu SA, Ihekwereme CP, Chukwu KI, Eluu SC, Azevedo RB. Formulation and evaluation of therapeutic potential of nanoemulsion of a blend of antimicrobial oils. Trop J Nat Prod Res. 2018; 2(2):67-73.
35. Liu Q, Huang H, Chen H, Lin J, Wang Q. Food-grade nanoemulsions: Preparation, stability and application in encapsulation of bioactive compounds. Molecules. 2019; 24(23):1-37.
36. Yudanti GP, Kuncahyo I, Ikasar ED. *In vitro* naringenin SNEDDS release test by dissolution. Nat Sci Eng Technol J. 2022; 2(1):79-84.
37. Yen C, Chen Y, Wu M-T, Wang C, Wu Y-T. Nanoemulsion as a strategy for improving the oral bioavailability and anti-inflammatory activity of andrographolide. Int J Nanomed. 2018; 13:669-680.
38. Penjuri SCB, Damineni S, Ravouru N, Poreddy SR. Self-emulsifying formulation of indomethacin with improved dissolution and oral absorption. Turkish J Pharm Sci. 2017; 14(2):108-119.
39. Mardhiani YD, Puriyani D, Fadilah L. Astaxanthin nanoemulsion formulation and evaluation. Indones J Pharm. 2022; 6(3):2-4.
40. Shiyan S, Suryani RP, Mulyani LN, Pratiwi G. Stability study of super saturable catechin-self nano emulsifying drug delivery system as antidiabetic therapy. Biointerface Res Appl Chem. 2022; 12(5):5811-5820.