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OPTIMIZATION OF NUTMEG FLESH (*Myristica fragrans*) EXTRACT NANOEMULSION IN A SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) USING THE D-OPTIMAL MIXTURE DESIGN METHOD FOR ANTI-INFLAMMATORY ACTIVITY

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Abstract
Background: Nutmeg flesh (*Myristica fragrans*) is known to contain flavonoid compounds with anti-inflammatory activity. The nutmeg flesh extract can be formulated into a nanoemulsion using the SNEDDS technique, an innovative drug delivery system that enhances drug absorption.
Objective: This study aimed to determine the optimal formulation of a nutmeg flesh (Myristica fragrans) nanoemulsion preparation using the SNEDDS technique as an anti-inflammatory agent.
Methods: This research employed an experimental design, with data collected through observation and measurement. Observations were conducted to evaluate the physical quality of the nanoemulsion formulation, as well as on the anti-inflammatory activity test using male white mice (Mus musculus).
Results: The analysis confirmed the presence of flavonoids as secondary metabolites. The optimal formula consisted of VCO (11%), Tween 80 (73%), and PEG 400 (15%), with predicted values of viscosity at 347 mPas, transmittance at 97.8%, emulsification time of 5 seconds, and a desirability value of 1. The anti-inflammatory activity, based on the percentage of inhibition, showed a high result in The SNEDDS test group showed high anti-inflammatory activity, with an inhibition rate of 90%.
Conclusions: The study revealed that the SNEDDS formulation of nutmeg flesh extract contained secondary metabolites and exhibited strong anti-inflammatory activity

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INTRODUCTION

Indonesia is one of the countries with a relatively high incidence of diseases involving inflammatory processes. The national p revalence of inflammatory-related diseases includes asthma (2.4%), joint disorders (7.3%), tumors (1.8%), hepatitis (1.2%), and cancer, which remains one of the leading causes of mortality in Indonesia (1). Inflammation can be managed pharmacologically; however, long-term administration of steroidal anti-inflammatory drugs often causes side effects, such as suppression of endogenous glucocorticoid synthesis, reduced immune response to infections, osteoporosis, moon face, hypertension, and gastrointestinal disorders (2). These side effects may be minimized through the use of alternative medicinal plants (3). The Taru Pramana manuscript is part of Balinese cultural heritage, representing traditional medical knowledge that serves as a foundation for complementary and integrative Balinese medicine (4).

The nutmeg flesh extract has potential as an anti-inflammatory agent in the *Usada Taru Pramana* manuscripts and has been used empirically in traditional therapy for its anti-inflammatory activity (5). The primary metabolite of nutmeg is myristicin, which is found in its fruit, seeds, and mace. Among these, the nutmeg flesh remains underutilized, although previous studies have shown that this part contains the highest concentration of sabinene compared to the seeds and mace (6). Nutmeg flesh extract has the potential as an anti-inflammatory agent because sabinene, flavonoids, can inhibit the production of prostaglandin (PGE2). The main challenges are not only related to solubility, but also to permeability and stability. Although flavonoids are hydrophilic compounds, many of them have low gastrointestinal membrane permeability and are prone to degradation, which results in low oral bioavailability. The nanoemulsion formulation can address these issues by protecting the compounds from degradation, increasing the surface contact with the gastrointestinal membrane, and improving absorption permeability (7).

The extract can be formulated into an oral dosage form, such as a nanoemulsion, to enhance drug absorption, particularly for lipophilic compounds (8). The Self-Nanoemulsifying Drug Delivery System (SNEDDS) is an innovative drug delivery approach designed to maximize absorption of lipophilic compounds. SNEDDS is an isotropic mixture of oil, surfactant, and co-surfactant, which, upon dilution with water under mild agitation, forms an oil-in-water nanoemulsion, thereby improving the solubility and bioavailability of poorly water-soluble drugs (9). The D-optimal mixture design can be applied in SNEDDS formulation to identify the optimal composition and achieve the desired characteristics. *Myristica fragrans* served as the active substance, SNEDDS was used as the delivery system to improve its absorption, and the D-optimal mixture design was employed to optimize the formulation. These three elements are therefore directly related within the research framework (10).

MATERIALS AND METHODS

Materials

Nutmeg flesh (*Myristica fragrans*) was obtained from Lalalinggah Village, Selemadeg Barat, Tabanan, Bali. The additional materials included 96% ethanol (Brataco), aquadest, Sulfuric acid (H_2SO_4), Mayer's and Dragendorff's reagent, Chloroform ($CHCl_3$), Acetic anhydride ($C_4H_6O_3$), Ferric chloride ($FeCl_3$) 10% solution, Virgin Coconut Oil (VCO), Tween 80 (Brataco), and PEG 400 (Brataco). Mice (*Mus musculus*), weighing 20–30 grams,

were used for the pharmacological anti-inflammatory test, with a total of 24 animals. Other materials used in the anti-inflammatory activity test included diclofenac sodium tablets (positive control), Na-CMC (negative control), and both the nutmeg flesh extract and its SNEDDS formulation (test groups).

Methods

Drying was performed using oven at 40–60°C until completely dry, then the material was ground into simplicia powder. The extraction of nutmeg flesh (*Myristica fragrans*) simplicia powder was carried out using the maceration method. The simplicia powder was placed in a container and soaked in 96% ethanol at a ratio of 1:10. The mixture was left to stand for three days with occasional stirring (at the same time each day for three days). The filtrate was then filtered to obtain the extract. The extract was concentrated using a rotary evaporator (Thermo Scientific Genesys, Amerika) at approximately 40°C until a thick concentrate was obtained. The concentrated extract was subsequently weighed to determine the yield.

Phytochemical screening of the nutmeg flesh (*Myristica fragrans*) extract was performed to identify secondary metabolites. The saponin test was carried out by adding distilled water to the simplicia powder in a test tube, shaking vigorously, and allowing it to stand for 15 minutes to observe foam formation. Flavonoid screening was conducted by extracting 0.5 g of sample with methanol under heating for 5 minutes, followed by the addition of 2–3 drops of hydrochloric acid and a small amount of magnesium powder. Alkaloids were detected by mixing the extract with 5 mL of sulfuric acid (H_2SO_4), shaking until two layers formed, and testing the acid layer with Mayer's and Dragendorff's reagents. Steroids and triterpenoids were identified by evaporating 2 ml of the test solution, dissolving the residue in chloroform, then adding acetic anhydride and concentrated sulfuric acid. Tannins were tested by adding 0.5 ml solution to a 10% ferric chloride ($FeCl_3$) solution, where a dark green or blue-black coloration indicated a positive result.

GC–MS analysis was also performed of the nutmeg flesh (*Myristica fragrans*) extract to identify the bioactive compounds responsible for anti-inflammatory activity. GC–MS analysis was performed using a Gas Chromatography–Mass Spectrometry (GC–MS) system (Agilent 7890B GC coupled with Agilent 5977A, USA) to identify the chemical constituents of the nutmeg flesh (*Myristica fragrans*) extract. Separation was carried out using an HP-5MS capillary column (30 m × 0.25 mm), helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The GC–MS method has been updated to include the column temperature program, total analysis time, and ion source conditions. The oven was initially set at 60 °C and held for 2 minutes, followed by a ramp of 10°C/min to 280°C, where it was held for 10 minutes. The total analysis time was 35 minutes. The ion source was operated in electron ionization (EI) mode at 70 eV, with a source temperature of 230°C. The mass spectra were recorded within a scan range of m/z 50–500.

The formulation of SNEDDS containing the nutmeg flesh (*Myristica fragrans*) extract was optimized using the D-optimal mixture design in Design-Expert 13 to determine the optimal composition of excipients. The extract was first dissolved in Virgin Coconut Oil (VCO) and mixed with a magnetic stirrer at 400 rpm for 5 minutes on a hotplate at 40 °C. This was followed by the addition of Tween 80 under the same conditions for 5 minutes, and subsequently, PEG 400 was added with continuous stirring for another 5 minutes. The mixture was then subjected to sonication for 15 minutes to obtain the final nanoemulsion

formulation. VCO was selected as the oil phase because it contains medium-chain triglycerides that promote spontaneous nanoemulsification and are suitable for enhancing oral absorption. Tween 80 was chosen as the surfactant due to its high HLB value and strong emulsifying capacity, while PEG 400 acted as the co-surfactant to lower interfacial tension and improve the stability of the droplets. These three components are frequently used together in SNEDDS formulations and have been demonstrated to produce stable systems with small globule size.

The SNEDDS formulation was characterized through several evaluations. Organoleptic properties were assessed by by 15 panelists through directly observing the physical appearance of the nanoemulsion, including its form, color, and odor. Phase separation was evaluated by centrifugation at 3500 rpm for 30 minutes. Percent transmittance was measured using a UV-Vis spectrophotometer at 650 nm with distilled water as the blank. pH measurement was conducted using a calibrated pH meter by immersing the electrode in the sample until the reading stabilized. Viscosity was determined using a viscometer. Emulsification time was assessed in distilled water, where the time required for the SNEDDS formulation to disperse completely and form a homogeneous nanoemulsion was recorded. The SNEDDS formulation was also evaluated for particle size, a particle size of less than 200 nm indicates that the formulation is in the nanoparticle or nanoemulsion range.

The in vivo anti-inflammatory study was conducted using healthy male white mice (*Mus musculus*), weighing 20–30 g and aged 2–3 months. A total of 24 mice were used and acclimatized for two weeks before experimentation. The anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in male white mice. Animals were randomly assigned to groups (n = 6). The extract was administered orally at doses of 50, 100, and 200 mg/kg in a vehicle (CMC Na 1%) at a dosing volume of 10 mL/kg. One hour after extract administration, acute inflammation was induced by subplantar injection of 0.1 mL of 1% (w/v) carrageenan in saline into the right hind paw. Percent inhibition of edema was calculated relative to the vehicle control group. Inflammation was induced on the mice's paw using carrageenan, a commonly employed inflammatory mediator. All experimental procedures involving animals were conducted in accordance with research ethics for animal research, the ethical approval number 285/EA/KEPK-BUB-2025. The animals were divided into four groups: a positive control (diclofenac sodium tablet), a negative control (Na-CMC 1%), and a test group (nanoemulsion formulation of nutmeg flesh extract and SNEDDS), all administered orally. The diameter of paw edema in mice was measured at 30-minute intervals following treatment. Data collected from observations were statistically analyzed using Analysis of Variance (ANOVA), followed by the Least Significant Difference (LSD) test.

RESULTS AND DISCUSSIONS

Nutmeg flesh powder (*Myristica fragrans*) was washed and extracted by maceration with 96% ethanol at a 1:10 ratio in an airtight container for three days, followed by two additional maceration steps until the extract lost its color. The filtrate was concentrated using a vacuum rotary evaporator at 40 °C to obtain a viscous ethanol extract. From 501 g

of starting material, the extract yield was 6.276%, with the total extraction process taking seven days (11).

Phytochemical screening of the nutmeg flesh (*Myristica fragrans*) extract revealed the presence of various secondary metabolite classes, including flavonoids, alkaloids, saponins, tannins, and triterpenoids. Flavonoids, in particular, have been widely reported in *Myristica fragrans* and are known to exhibit anti-inflammatory activity. They can inhibit cyclooxygenase and lipoxygenase enzymes, as well as suppress the production of pro-inflammatory cytokines (12).

Table 1. Phytochemical profile of the nutmeg flesh (*Myristica fragrans*) extract

No.	Target compounds	Reagent	Presence in the extract
1	Flavonoids	NaOH 10%	(+)
		Mg dan HCl	(+)
2	Alkaloid	Mayer	(+)
		Dragendorff	(+)
		Bouchardat	(+)
3	Saponins	Aquadest	(+)
4	Tannin and Polifenol	FeCl 10%	(+)
5	Steroids and terpenoids	Kloroform, asam asetat, asam sulfat	(+) terpenoid (-) steroids

Description: (+) Positive: There are secondary metabolite compounds
(-) Negative: There are no secondary metabolite compounds

GC-MS analysis also detected alpha-terpinene in the extract fraction, although its quantity was lower than that of sabinene. Nevertheless, alpha-terpinene has been reported to possess significant anti-inflammatory activity (13).

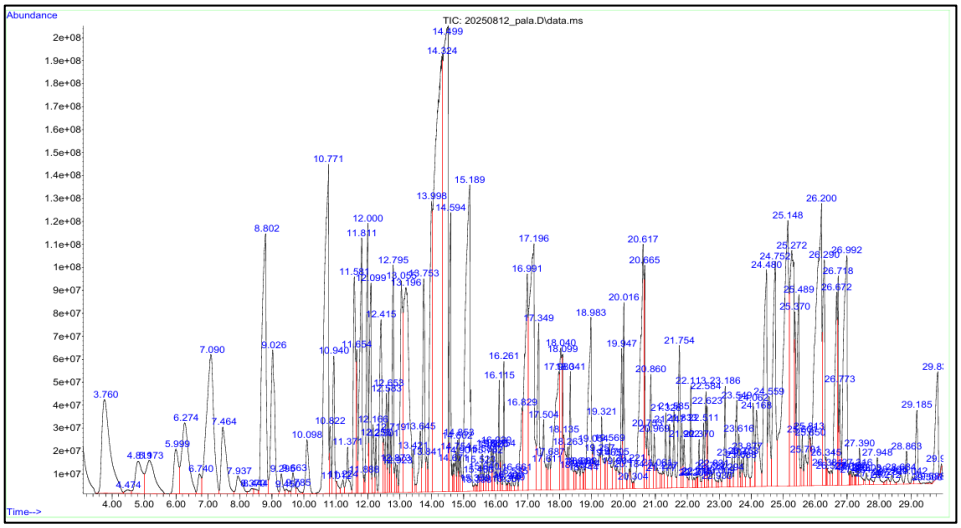

Figure 1. GC-MS Analysis Results

Table 2. GC-MS Analysis

No.	Retention Time (min)	Compound Name	Molecular Formula	Peak Area (%)	Possible Activity
1	14.76	α-Pinene	$C_{10}H_{16}$	6,3	Anti-inflammatory
2	12.47	Sabinene	$C_{10}H_{16}$	8,7	Anti-inflammatory
3	11.85	Myristicin	$C_{11}H_{12}O_3$	10,2	Anti-inflammatory
4	10.87	Eugenol	$C_{11}H_{12}O_2$	13,1	Anti-inflammatory
5	9.45	Methyleuhenol	$C_{11}H_{14}O_2$	18,5	Anti-inflammatory

The formulation was developed using the D-optimal mixture design in Design Expert *13*. The d-optimal mixture design is an experimental design method used to optimize self-nanoemulsifying drug delivery systems (SNEDDS) and determine the ideal composition of multiple components to achieve the best formulation. Upper and lower limits were set to define the minimum and maximum concentrations of the SNEDDS components.

Table 3. Upper and Lower Percentage Limits of VCO: Tween 80: PEG 400

No.	Components	Lower Limits	Upper Limits
1	VCO	11,1%	20%
2	Tween 80	60%	77,7%
3	PEG 400	11,1%	20%

Viscosity measurement, emulsification time in simulated gastric fluid, and percent transmittance were used as primary evaluation parameters in the optimization of SNEDDS formulations using Design-Expert *13*. Percent transmittance indicates the amount of light, that passes through the sample, as measured with a UV-Vis spectrophotometer. A high percent transmittance reflects a clear and transparent formulation, indicating small droplets that are well-dispersed. In contrast, a low percent transmittance suggests a turbid formulation with larger droplets or aggregation. Emulsification time is defined as the duration required for a formulation, particularly SNEDDS or self-emulsifying systems, to form a stable nanoemulsion upon contact with a liquid medium, such as simulated gastric fluid, under mild agitation. This parameter reflects how quickly the formulation can disperse homogeneously. Ideally, SNEDDS formulations should have low viscosity to allow rapid dispersion upon contact with gastric fluid; high viscosity results in longer emulsification times (14).

Table 4. Formula Run Results Obtained from Design Expert

	Component 1	Component 2	Component 3	Response 1	Response 2	Response 3
Run	**A:VCO**	**B:Tween 80**	**C:PEG 400**	**Viscosity**	**Transmittance**	**Emulsification**
	%	**%**	**%**	**mPas**	**%**	**Second**
1	19.6	66.2	14.2	356	4.35	39.9
2	11.1	68.9	20	307	97.3	9.7
3	15.7	67.7	16.6	324	96	43.2
4	20	60	20	282	28.5	8.1
5	11.6	77.3	11.1	374	96.4	10.0
6	20	68.9	11.1	323	94.7	81.7
7	15.2	73.7	11.1	349	97.8	59.8
8	16.7	63.3	20	345	30.4	7.0
9	20	62.9	17.1	303	65.9	11.0
10	14.5	71	14.5	355	94.4	38.9
11	11.1	73.6	15.3	342	97.7	8.4

The analysis of viscosity measurement data using ANOVA revealed that the d-optimal mixture method identified the linear model as the best fit, outperforming both quadratic and cubic models. This selection was based on the linear model, which yielded an F-value of 4.62 with a p-value of 0.0464 (<0.05), indicating that the model is statistically significant at a 95% confidence level. In other words, the composition of the mixture has a significant effect on the viscosity response. The ANOVA results for viscosity measurements are presented in Figure 2, obtained from data processing using Design Expert version 13.

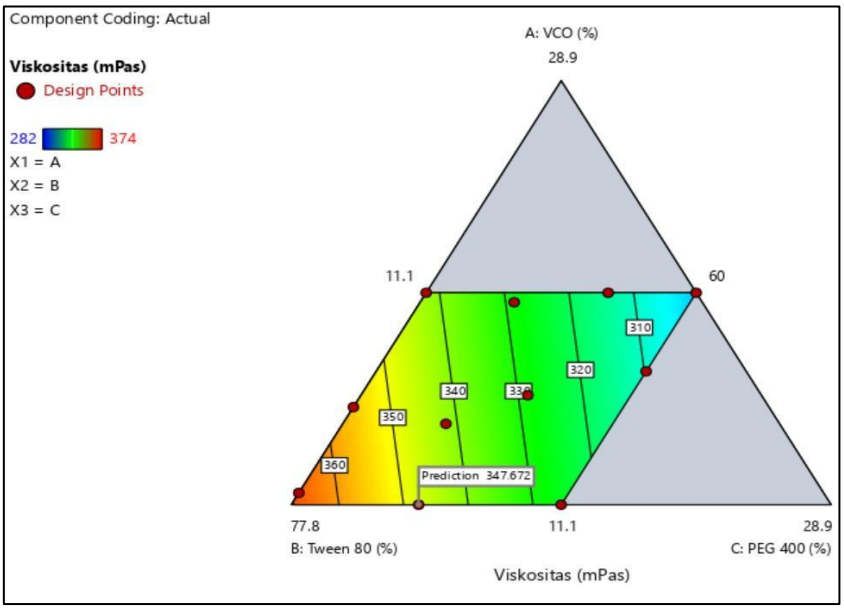

Figure 2. Linear Model for Viscosity

Viscosity testing, as shown in Figure 2, indicated that the residuals of viscosity closely followed a straight line, suggesting that the residuals were normally distributed (15). The results showed that the viscosity of the SNEDDS formulations ranged from 282 to 374 mPa · s, with an optimal predicted value of 347.6 mPa · s. An increase in tween 80 concentration tended to increase viscosity, whereas PEG 400, as a cosurfactant, was able to reduce viscosity by enhancing the fluidity of the system. Previous studies reported a viscosity of 356.7 ± 1.53 mPas for SNEDDS containing ethanol *Clinacanthus nutans* leaves extract, indicating that the dominance of tween 80 significantly increased viscosity (16). Other research has also shown that higher surfactant fractions in SNEDDS formulations contribute to increased viscosity due to the formation of a thicker interfacial layer (17). Therefore, the viscosity values obtained in this study fall within the ideal range, allowing for a balance between emulsification rate and dispersion stability.

The analysis of emulsification time data using ANOVA revealed that the D-optimal mixture method identified the quadratic model as the best fit, surpassing both the linear and cubic models. This selection was based on the quadratic model, which yielded an F-value of 10.74 with a p-value of 0.0105 (<0.05), indicating that the model is statistically significant. In other words, the composition of the mixture has a significant effect on the response time of emulsification (18). The residual error was 557.90, which is relatively small compared to the total variation of 6548.65, suggesting that the model adequately explains the data. The ANOVA results for emulsification time measurements are presented in Figure 3, obtained from data processing using Design Expert version 13.

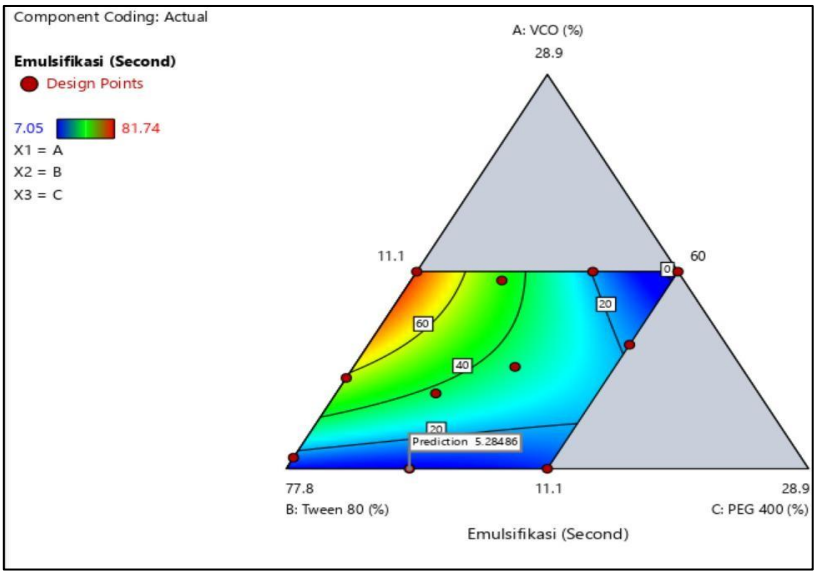

Figure 3. Quadratic Model for Emulsification Time

The results of this study indicate that increasing the concentration of Tween 80 significantly accelerates the emulsification time of SNEDDS. In contrast, a high oil fraction tends to slow down the emulsification process, and PEG 400 acts as a supporting co-surfactant. The comparison among VCO, tween 80, and PEG 400 showed that, despite differences in oil type and active ingredient properties, the pattern of surfactant dominance in accelerating emulsification remained consistent. This highlights that selecting the

appropriate surfactant concentration is a key factor in producing SNEDDS with rapid emulsification and favorable physical characteristics.

The analysis of % transmittance data using ANOVA revealed that the D-optimal mixture method identified the cubic model as the best fit, surpassing both the linear and quadratic models. This selection was based on the cubic model, which yielded an F-value of 8.14 with a p-value of 0.0215 (<0.05), indicating that the model is statistically significant. In other words, the composition of the mixture has a significant effect on the response % transmittance. The ANOVA results for % transmittance measurements are presented in Figure 4, obtained from data processing using Design Expert version 13.

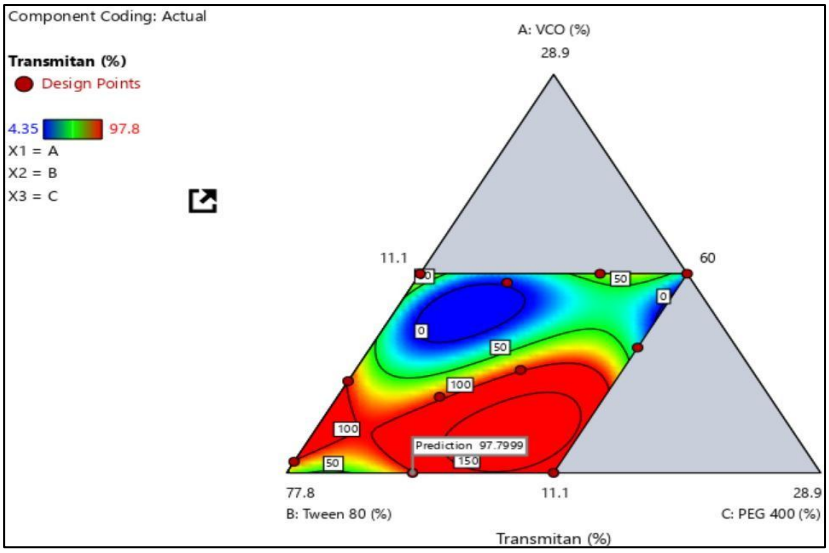

Figure 4. Cubic Model for % Transmittance

The results of this study showed that the highest % transmittance reached 97.8% in regions with a high proportion of Tween 80 surfactant and a relatively low oil (VCO) fraction. This condition indicates the formation of a clear nanoemulsion with a small droplet size and homogeneous distribution, as the system allows nearly all light to pass through. Therefore, %transmittance can be considered a key indicator of successful nanoemulsion formation in SNEDDS systems, with values greater than 90% representing good dispersion quality and meeting the SNEDDS grade A criteria.

Based on the optimization settings, the suggested formulation was identified as the optimal formula, along with the predicted response values for viscosity, emulsification time, and % transmittance of the SNEDDS nutmeg flesh extract nanoemulsion, as shown in Table 4. Among the three responses, emulsification time was prioritized as the primary determinant of the best formulation and was therefore assigned the highest weighting (+++++ importance). The ideal value is <1 minute, as this indicates the ability to form a stable and precise nanoemulsion rapidly. A % transmittance value approaching 100% reflects that the particles within the formulation have reached the nanometer range. The transmittance response was assigned a weighting of (+++), with the expectation that the optimal formula produced in this study would achieve the highest transmittance value, approaching the target of 97.8%. Meanwhile, viscosity was set within the target range (importance +++), with a lower limit of 282 mPa ·s and an upper limit of 374 mPa ·s.

Table 5. Optimal Formula Settings

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:VCO	is in range	11.1	20	1	1	3
B:Tween 80	is in range	60	77.7	1	1	3
C:PEG 400	is in range	11.1	20	1	1	3
Viscosity	is in range	282	374	1	1	3
Transmittance	is target = 97.8	4.35	97.8	1	1	3
Emulsification	minimize	7.05	81.74	1	1	5

Based on these setting formulas, one optimal formula was obtained, as presented in Table 4, with a desirability value of 1.000. The desirability index ranges from 0 to 1, where values closer to 1 indicate that the formula better meets the targeted response criteria. This optimal formula was subsequently evaluated for viscosity, emulsification time, and %transmittance**.**

Table 6. Optimal Formula and Predicted Response Values of SNEDDS

Run	VCO (%)	Tween 80 (%)	PEG 400 (%)	Viscosity (mPas)	Transmittance (%)	Emulsification (second)	Desirability
1	11.110	73.597	15.293	347.672	97.800	5.285	1.000

Table 7. Optimal Formula Evaluations

Run	Organoleptic			pH	Homogenity
	Form	Color	Odor		
1	Semisolid	Light yellow	Strong	6.0	Homogeneous

The viscosity test data of the optimal SNEDDS formula containing nutmeg (Myristica fragrans) extract were analyzed using ANOVA with IBM SPSS Statistics version 27. Verification was carried out by comparing the predicted viscosity values obtained from Design Expert 13 software with the observed values from laboratory experiments. Data processing with IBM SPSS Statistics version 27 produced a probability value of 0.000. Since this probability value was less than $\alpha = 0.05$, it can be concluded that there is a significant difference between the predicted viscosity values from the design expert and the observed laboratory results as presented in Table 8.

Table 8. Viscosity Evaluation of the Optimal SNEDDS Formula

Parameter (Test Type)	Predicted Value (mPas)	Experimental Value (mPas)			Mean Value ± SD	*p-value*
		Replicate 1	Replicate 2	Replicate 3		
Viscosity	347.672	132	132	133	132. 333 ± 2.07	0.000

The emulsification time test data of the optimal SNEDDS formula containing nutmeg (Myristica fragrans) extract were analyzed using ANOVA with IBM SPSS Statistics version 27. Verification was carried out by comparing the predicted emulsification time values obtained from Design Expert 13 software with the observed values from laboratory experiments. Data processing with IBM SPSS Statistics version 27 produced a probability value of 0.478. Since the probability value was greater than α = 0.05, it can be concluded that there is no significant difference between the predicted emulsification time values from Design Expert and the observed laboratory results, as presented in Table 9.

Table 9. Emulsification Evaluation of the Optimal SNEDDS Formula

Parameter (Test Type)	Predicted Value (second)	Experimental Value (second)			Mean Value ± SD	*p-value*
		Replicate 1	Replicate 2	Replicate 3		
Emulsification	5.285	5.39	6.31	5.89	5,863 ± 0.35	0.478

The %transmittance test data of the optimal SNEDDS formula containing nutmeg (Myristica fragrans) extract were analyzed using ANOVA with IBM SPSS Statistics version 27. Verification was performed by comparing the predicted %transmittance values obtained from Design Expert 13 software with the observed values from laboratory experiments. Data processing with IBM SPSS Statistics version 27 produced a probability value of 0.286. Since this probability value was greater than α = 0.05, it can be concluded that there is no significant difference between the predicted % transmittance values from the design expert and the observed laboratory results, as presented in Table 10.

Table 10. %Transmittance Evaluation of the Optimal SNEDDS Formula

Parameter (Test Type)	Predicted Value (%)	Experimental Value (%)			Mean Value ± SD	*p-value*
		Replicate 1	Replicate 2	Replicate 3		
Transmittance	97.800	97.100	96.900	97.600	97.200 ± 0.40	0.286

Based on the Particle Size Analysis (PSA) results, as shown in Figure 5, the particle size of the SNEDDS formulation was found to be within the range of 10–60 nm. The particle size of the optimal formula met the requirements for nanoemulsions, as it was below 200 nm. The reduction in particle size occurred due to longer stirring duration; the longer the stirring time, the smaller the resulting particle size, as more particles are broken down into nanosized dimensions (19).

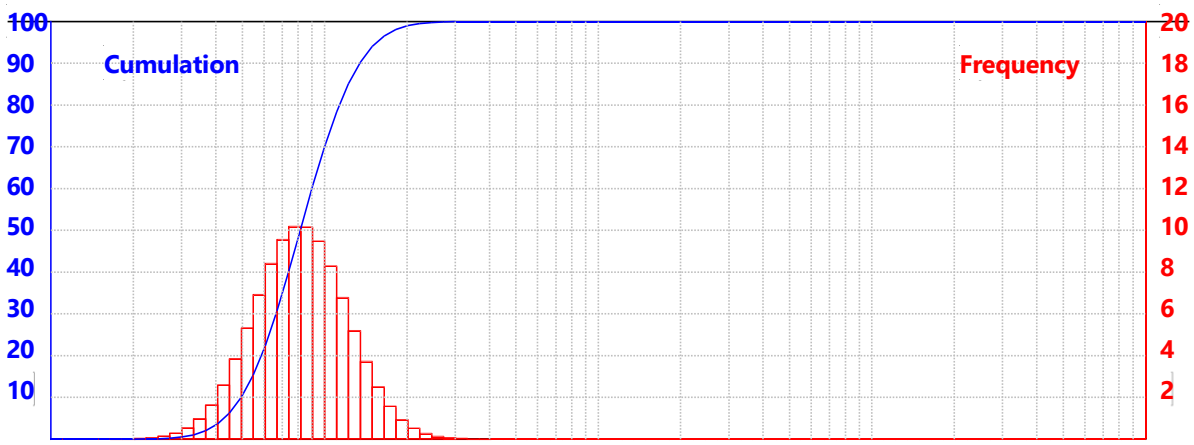

Figure 5. Particle Size Analysis (PSA) SNEDDS

The in vivo anti-inflammatory activity test using male white mice demonstrated different responses among treatment groups. The negative control group (Na-CMC 1%) exhibited no edema-inhibiting effect, with the percentage of inhibition remaining at 0% throughout the observation period. In contrast, the positive control group (Na-diclofenac) showed the strongest edema inhibition effect, reaching approximately 93% at 90 minutes (20).

The nutmeg flesh extract group showed anti-inflammatory activity, with % inhibition ranging from 48% to 66% up to the 90th minute, indicating a moderate anti-inflammatory effect. Interestingly, the SNEDDS nutmeg flesh extract group demonstrated a higher effect compared to the conventional extract, with %inhibition approaching 77% at 90 minutes. These findings confirm that the SNEDDS formulation significantly enhances the anti-inflammatory activity of nutmeg flesh extract (21). This enhancement can be attributed to the SNEDDS system's ability to improve the solubility, dissolution, and bioavailability of the active compounds contained in the extract. Therefore, it can be concluded that nutmeg flesh extract possesses promising anti-inflammatory potential, and formulation innovation using SNEDDS plays a crucial role in enhancing its pharmacological effectiveness (22).

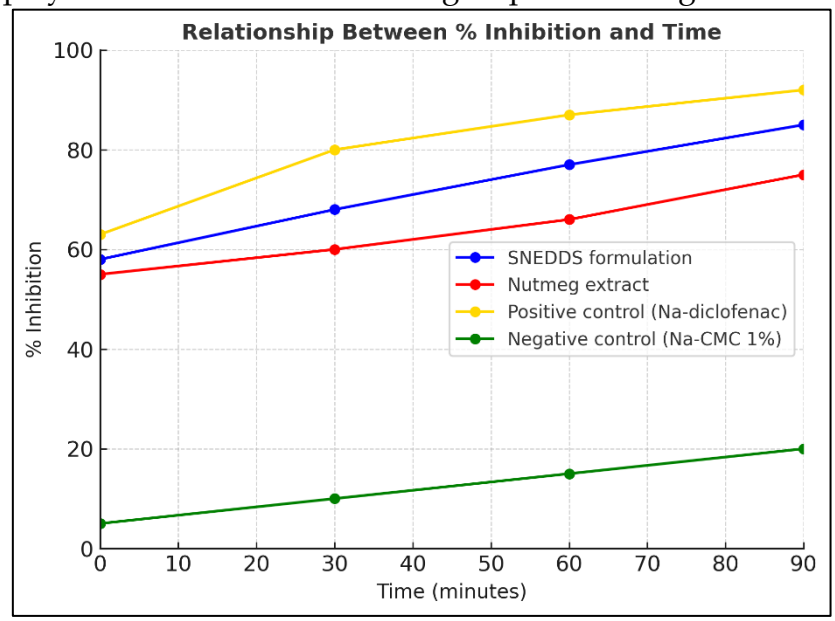

Figure 6. Average Percentage of Edema Inhibition in Each Group

The statistical analysis of each group through normality and homogeneity tests showed P>0.05, indicating that the data were normally distributed and homogeneous. Since the data met these assumptions, the analysis was continued using ANOVA and LSD tests with a 95% confidence level (23). The results of the LSD test revealed that the positive control group (Na-diclofenac), the SNEDDS test group, and the EDBH test group were significantly different from the negative control group (Na-CMC 1%) with a significance value of P=0.000 (P<0.05). Meanwhile, the positive control group (Na-diclofenac), the SNEDDS test group, and the EDBH test group also showed P<0.05, indicating that all three groups exhibited significant anti-inflammatory effects. The findings of this study are in agreement with previously reported data on the anti-inflammatory activity of *Myristica fragrans* and its major volatile constituents. Several studies have demonstrated that monoterpenes such as sabinene exert anti-inflammatory effects through the inhibition of pro-inflammatory mediators and modulation of oxidative stress. These findings support the hypothesis that the bioactive compounds present in the extract contribute to the reduction of edema observed in the carrageenan-induced paw model (24).

Furthermore, the enhanced performance of the SNEDDS formulation is consistent with reports from recent literature showing that nanoemulsion-based delivery systems improve the oral bioavailability of poorly absorbed natural compounds. Previous studies have noted that SNEDDS formulations increase surface area, reduce globule size, and facilitate more efficient absorption through the gastrointestinal tract. Similar formulations have been shown to produce faster onset and stronger anti-inflammatory responses when compared with crude extracts or conventional dosage forms (25).

Overall, the findings of this research are consistent with the existing body of evidence, indicating that both the extract and its SNEDDS formulation possess anti-inflammatory activity. However, the superior efficacy of SNEDDS highlights the importance of delivery systems in maximizing therapeutic outcomes for plant-derived compounds.

CLINICAL IMPLICATION

The clinical implications of this study provide preliminary information on the secondary metabolite content of traditional plants that have been optimally formulated into SNEDDS as anti-inflammatory agents.

LIMITATIONS

The limitations of this study include the use of a limited number of samples, which results in fewer extracts.

CONCLUSIONS

This study demonstrated that the nutmeg flesh (*Myristica fragrans*) extract contains active secondary metabolites such as flavonoids, sabinene, and α-terpinene with anti-inflammatory potential, and its formulation into SNEDDS optimized using the D-optimal mixture design produced a stable nanoemulsion with ideal viscosity, rapid emulsification time, high transmittance, and particle size within the nanometer range. In vivo results confirmed that the SNEDDS formulation significantly enhanced the anti-inflammatory

activity of the extract compared to the conventional form, approaching the efficacy of the positive control, thus highlighting its potential as a promising natural anti-inflammatory agent.

CONFLICT OF INTEREST

The research was conducted without commercial or financial relationships that could be construed as a potential conflict of interest.

AUTOR CONTRIBUTIONS

M.D.S. designed and conducted the research, analyzed and interpreted the data, and wrote the manuscript draft. R.K. conducted the research and wrote the draft of the manuscript. M. V conducted the research.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors used ChatGPT (OpenAI, version GPT-5) to assist in improving the language and grammar of the manuscript. The authors reviewed and verified the content to ensure accuracy and integrity.

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