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OPTIMIZATION OF NANO SPRAY GEL FROM FERMENTED RED GINGER EXTRACT FOR ANTI-INFLAMMATORY

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Red ginger, Fermentation, Simplex Lattice Design, Nano spray gel

Abstract

Background: Red ginger contains bioactive compounds such as gingerol and shogaol with known anti-inflammatory activity, making it a potential active ingredient for nanospray gel formulations.
Objective: This study aimed to optimize a nano spray gel formulation containing fermented red ginger extract using the Simplex Lattice Design (SLD).
Methods: An experimental laboratory study was performed. Formulation optimization was conducted using Design-Expert® version 13 with the Simplex Lattice Design (SLD) approach by varying the concentrations of Carbopol 940, Triethanolamine (TEA), and Tween 80. The responses evaluated were percent transmittance, pH, and viscosity. Anti-inflammatory activity was assessed in vivo in male mice using a posttest-only control group design. The treatment groups included sodium diclofenac as the positive control (KP), CMC-Na as the negative control (KN), 0.5% red ginger extract (EJM), 0.5% fermented red ginger extract (EFJM), and a nano spray gel containing 0.5% fermented red ginger extract (SNSG)
Result: The optimized formulation achieved a desirability value of 0.948. The formulation exhibited a pH of 6.0 ± 0.06, transmittance of 99.1 ± 0.12%, and viscosity of 537.7 ± 2.08 mPa·s. Particle size analysis showed an average particle size of 14.35 nm and a PDI of 0.1012, confirming the successful development of a homogeneous nanodispersion system. The nano spray gel containing fermented red ginger extract demonstrated notable anti-inflammatory activity, with an AUC value of 0.0145 and an AIA of 80.67% after 1.5 hours of treatment, indicating effective inhibition of inflammation

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Conclusions: The SLD method effectively optimized the nanospray gel formulation from fermented red ginger extract, and demonstrated potential as a natural-based topical anti-inflammatory product.

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INTRODUCTION

Red ginger (*Zingiber officinale* var. *rubrum*) is a medicinal plant classified as a rhizomatous herb. It is widely recognized for its high content of bioactive compounds, particularly flavonoids and phenolic compounds, which exhibit potent antioxidant and anti-inflammatory activities (1). The concentration of bioactive compounds in red ginger (*Zingiber officinale* var. *rubrum*) rhizomes can be enhanced through fermentation. This was evidenced by an increase in extract yield following fermentation, from 8.21% to 14.30%. Gas chromatography–mass spectrometry (GC–MS) analysis further demonstrated a marked increase in the relative abundance of the principal bioactive constituents, with 6-gingerol increasing from 1.23% to 5.43% and 6-shogaol from 5.25% to 13.36%. These findings indicate that fermentation prior to extraction effectively enhances both the yield and the relative abundance of secondary metabolites, thereby improving the overall phytochemical quality of red ginger extracts (2). The enhancement is likely attributable to microbial activity during fermentation, which can stimulate the biosynthesis and biotransformation of secondary metabolites, thereby increasing the accumulation of bioactive compounds (3). These characteristics highlight red ginger (*Zingiber officinale* var. *rubrum*) as a promising source of bioactive compounds for the development of natural-based pharmaceutical formulations. However, the clinical application of phytochemicals is often limited by their poor dermal penetration and bioavailability. To address these limitations, nanoparticle-based delivery systems, particularly nanogels, have been extensively investigated owing to their ability to improve the stability, skin permeation, and controlled release of bioactive compounds (4). Among these systems, nano spray gel has emerged as an innovative topical dosage form that enables convenient, uniform, and non-invasive administration while facilitating efficient delivery of therapeutic agents to the skin. Incorporation of red ginger extract into a nano spray gel is therefore expected to enhance the cutaneous delivery of its bioactive constituents and improve its therapeutic potential as a natural anti-inflammatory agent (5).

The nano spray gel formulation was optimized using Carbopol 940, Tween 80, and triethanolamine (TEA) as the primary excipients. Carbopol 940 serves as the gelling agent, providing the viscosity and rheological properties required for effective sprayability and prolonged skin retention. Tween 80 functions as a nonionic surfactant that enhances the solubilization and homogeneous dispersion of hydrophobic constituents while contributing to nanoparticle stability. TEA acts as a neutralizing agent by ionizing the carboxylic acid groups of Carbopol 940, thereby promoting gel formation while maintaining the formulation pH within the physiological range for the skin, ensuring skin compatibility. Collectively, these excipients govern the physicochemical characteristics of the nano spray gel, including viscosity, pH, physical stability, and drug release profile, which are critical determinants of formulation performance. Optimization of these formulation attributes is therefore essential, achieving a stable and effective delivery system with the potential to improve the dermal bioavailability of the encapsulated bioactive compounds (6).

Nanoparticle-based systems, such as nanogels, can enhance the distribution, absorption, and penetration of active compounds across the skin barrier. Spray gel is an innovative dosage form that allows for easy and uniform topical application through spray packaging. A nano spray gel containing red ginger extract offers potential as a natural anti-inflammatory agent, due to its ability to enhance the delivery of active compounds to the skin (7). Previous studies have demonstrated that ginger rhizomes possess strong anti-

inflammatory effects, primarily due to compounds such as gingerols and shogaols (2). These constituents exert their effects by modulating inflammatory pathways, including the inhibition of key enzymes such as cyclooxygenase-2 (COX-2) and lipoxygenase, as well as suppressing the production of pro-inflammatory mediators, including prostaglandins and leukotrienes (8).

In developing the fermented red ginger nano spray gel, formulation optimization was performed using the Simplex Lattice Design (SLD) method through Design Expert 13 software. This statistical approach was employed to explore the optimal combination of Carbopol, Triethanolamine (TEA), and Tween 80 as the gel base components. The SLD method generated nine formulation alternatives to evaluate with the primary objective of identifying a composition that best meets the desired physicochemical properties (9).

This study introduced a fermentation process prior to extraction to enhance the biological activity and secondary metabolite content of red ginger rhizomes. The fermented extract was subsequently formulated into a nano spray gel to improve bioavailability. The novelty of this research lies in combining fermentation technology with the optimization of a nanotechnology-based spray gel formulation using the Simplex Lattice Design (SLD) approach.

MATERIALS AND METHODS

The research employed a laboratory experimental design, conducted at the Pharmacy Laboratory of the Bintang Persada Institute of Technology and Health. The plant material used in this study was red ginger (*Zingiber officinale* var. *rubrum*), sourced from Nongan Village, Rendang District, Karangasem, Bali. The plant material was taxonomically authenticated at the Bandung Institute of Technology, West Java, Indonesia (No. 6324/IT1.C11.2/TA.00/2024). The experimental stages included the rejuvenation of *Trichoderma harzianum* mold, fermentation, extraction, formulation, physical quality evaluation of the preparation, and particle size analysis of the nanodispersion formulation. Optimization of the fermented red ginger nanospray gel formulation was carried out, using the Simplex Lattice Design (SLD) approach through DesignExpert 13 software. The optimized gel base components consisted of Carbopol 940, Triethanolamine (TEA), and Tween 80, with optimization parameters including % transmittance, pH, and viscosity. The anti-inflammatory activity of fermented red ginger extract was evaluated in vivo. Twenty-five male white mice (*Mus musculus*) were employed as experimental animals. The study protocol was reviewed and approved by the Research Ethics Committee of the Bali Institute of Technology and Health (Approval No. 04.0373/KEPITEKES-BALI/X/2024), and all procedures were conducted in accordance with applicable ethical guidelines for animal research. The equipment used in this study included: mortar and pestle, glassware, dropper pipettes, horn spoons, filter paper, parchment paper, aluminum foil, knives, simple fermenters, measuring flasks, blenders, rotary evaporators (BUCHI), hot plate stirrers (Thermo Scientific), cups, pH meters (YIERYI), viscometers (NDJ-5S), stopwatches, UV-Vis spectrophotometers (Thermo Scientific Genesys), centrifuges (80-2B), Laminar Air Flow (BioBase), autoclaves (GEA YX-18LM), incubators (Memmert), analytical balances (Kenko), particle size analyzers (Biobase BK-802N), Plethysmometer, and spray bottles. The materials used included red ginger extract, Potato Dextrose Agar (PDA), potatoes, *Trichoderma harzianum*, 96% ethanol, dextrose, methyl paraben, Tween 80, Carbopol 940, sorbitol, propyl

paraben, distilled water, Triethanolamine (TEA), aquabidest, carrageenan, diclofenac sodium gel, CMC-Na, and 70% alcohol.

Red Ginger Fermentation:

The fermentation process of red ginger powder was conducted under aerobic conditions. A total of 100.0747 grams of red ginger powder was accurately weighed and inoculated with *Trichoderma harzianum* mold using Potato Dextrose Broth (PDB) as the growth medium. During fermentation, the sample was maintained at a moisture content of 40–45% and a pH of 4, and incubated under aerobic conditions for 6 days (10).

Preparation of Fermented Red Ginger Extract:

The fermented red ginger powder was extracted using the maceration method with 96% ethanol at a ratio of 1:5 (w/v). The maceration process involved initial stirring for 30 minutes, followed by static maceration for 72 hours. Afterward, the sample underwent re-maceration for an additional 48 hours under the same conditions. The resulting filtrate was then concentrated using a rotary vacuum evaporator to obtain a thick extract of fermented red ginger (2).

Nano Spray Gel Formulation :

Optimization of the nano spray gel formulation containing fermented red ginger extract (EFJM) was carried out using the Simplex Lattice Design (SLD) method, implemented through Design Expert version 13 software. This software facilitated the statistical design of formulation combinations to determine the optimal composition of Carbopol 940, Triethanolamine (TEA), and Tween 80 as gel base components. The formulation design followed the simplex lattice design model, where the response variables evaluated included percent transmittance, pH, and viscosity. The composition of the nano spray gel formulation containing fermented red ginger extract is summarized in Table 1.

Table 1. the composition of the nano spray gel formulation containing fermented red ginger extract

Formulation						
Carbopol 940	**TEA**	**Tween 80**	**Sorbitol**	**Methyl paraben**	**Propyl paraben**	**Aquadest**
0.1-0.82	0.08-0.56	0.1-0.82	1.2	0.2	0.04	add 100

As shown in Table 1, the concentrations of Carbopol 940, triethanolamine (TEA), and Tween 80 were systematically varied in each experimental run according to the simplex lattice design. In contrast, the concentrations of sorbitol, methylparaben, propylparaben, and distilled water were kept constant throughout all formulations. Consequently, the optimization of the gel base focused on evaluating the effects of Carbopol 940, TEA, and Tween 80 on the physicochemical properties of the nano spray gel.

This experimental design approach was employed to identify the most effective combination of gel base ingredients for optimizing the EFJM nano spray gel formulation. The concentrations of the formulation components were adjusted according to the experimental design generated by the Simplex Lattice Design (SLD), as shown in Table 2.

Table 2. Concentrations of the formulation component

Formulation	Component Design		
Run	Component 1 A:Carbopol 940 (%)	Component 2 B: TEA (%)	Component 3 C: Tween 80 (%)
1	0.46	0.44	0.1
2	0.58	0.2	0.22
3	0.1	0.08	0.82
4	0.1	0.8	0.1
5	0.46	0.44	0.1
6	0.82	0.08	0.1
7	0.34	0.32	0.34
8	0.1	0.08	0.82
9	0.46	0.08	0.46
10	0.46	0.08	0.46
11	0.1	0.44	0.46
12	0.82	0.08	0.1
13	0.22	0.56	0.22
14	0.1	0.8	0.1
15	0.22	0.2	0.58

Gel Base Preparation: Carbopol 940 was dispersed in warm distilled water and allowed to swell completely. The mixture was then stirred until homogeneous, followed by the gradual addition of triethanolamine (TEA) to neutralize the pH and improve the consistency of the gel base (11).

Nanodispersion Preparation: The nanodispersion was prepared by mixing Tween 80, methylparaben, propylparaben, fermented red ginger extract, and sorbitol until a homogeneous mixture was obtained, using an overhead stirrer operated at 600 rpm for 30 minutes (12). The resulting nanodispersion was subsequently combined with the pre-prepared gel base and stirred again using the same overhead stirrer at 600 rpm for 15 minutes, following the composition proportions determined by the Simplex Lattice Design (SLD).

Physical Quality Testing of Nano Spray Gel Preparations:

The % transmittance test was conducted to assess the clarity of the nano spray gel. A transmittance value approaching 100% indicates that the particle size is within the nanometer range, confirming that the dispersion is clear and well-distributed (13). The pH test was conducted to evaluate the acidity level of the preparation and to ensure its safety for topical application. Viscosity testing was performed to evaluate the spraying ability and physical stability of the nano spray gel (11). The phase separation or stability test was used to assess the formulation's resistance to physical stress (14). The homogeneity test aimed to evaluate the uniform distribution of particles within the gel (15). The adhesive spreadability test was performed by spraying the nano spray gel onto the upper arm from a distance of 3 cm. Good adhesion is indicated by the formulation remaining on the skin surface without dripping (16). The drying time test was conducted by applying the nano spray gel to the

forearm and measuring the time for it to dry completely (17). The spray pattern test was conducted by spraying the nano spray gel onto a mica plastic surface from a distance of 5 cm, in accordance with standard testing protocols (15). The organoleptic evaluation was performed by assessing the physical characteristics of the preparation through visual and olfactory observation, focusing on color, odor, and texture. Ideal organoleptic attributes for a spray gel formulation include a clear or transparent appearance, absence of turbidity, and being free from visible air bubbles (18).

In Vivo Anti-Inflammatory Activity Test of the Extract:

The anti-inflammatory activity of red ginger extract was evaluated in vivo using a plethysmometer by measuring the paw volume of male mice. A posttest-only control group design was adopted. A total of 25 male mice were equally distributed into five experimental groups (n = 5 per group): the negative control group treated with CMC-Na (KN), the positive control group treated with sodium diclofenac (KP), the red ginger extract group treated with 0.5% extract (EJM), the fermented red ginger extract group treated with 0.5% extract (EFJM), and the nano spray gel group treated with a formulation containing 0.5% fermented red ginger extract as the active ingredient. Paw inflammation was induced with 1% carrageenan. Measurements were conducted at baseline and at 30, 60, and 90 minutes post-induction (19). The anti-inflammatory effect was assessed based on two parameters: the area under the curve (AUC) and the percentage of anti-inflammatory activity (%AIA). The AUC was determined using the trapezoidal method to quantify the overall inflammatory response over time, as described by the following equation :

$$AUCn = \frac{(V_{i} + V_{i-1})}{2} x\ t$$

The percentage of anti-inflammatory activity (%AIA) was determined by comparing the AUC of the treatment group with that of the negative control, according to the following formula:

$$\%AIA = \frac{AUC\ negative\ control - AUC\ treatment}{AUC\ negative\ control} x\ 100\%$$

The area under the curve (AUC) was determined to quantify the cumulative inflammatory response over the observation period. In anti-inflammatory evaluations, a lower AUC value indicates a reduction in overall edema, reflecting enhanced anti-inflammatory efficacy. A higher percentage of anti-inflammatory activity (%AIA) corresponds to a greater anti-inflammatory effect of the tested compound. Typically, %AIA values of 50% or greater are considered indicative of strong activity, values between 25% and 50% indicate moderate activity, and values below 25% represent weak activity (20).

RESULTS AND DISCUSSIONS

The simplicia fermented for six days were extracted using the maceration method with 96% ethanol as the solvent. The resulting macerate was then filtered, and the filtrate was concentrated using a rotary evaporator. From this process, a thick extract of fermented red ginger (EFJM) weighing 13.0650 grams was obtained, yielding an extraction efficiency

of 13.06%. This yield meets the standard for a good extract, which is generally considered acceptable if it exceeds 10% (21). The obtained EFJM extract was then utilized as the active ingredient in the nano spray gel formulation. The formulation was developed based on the composition pattern determined by the Simplex Lattice Design (SLD) model. The actual design included the components Carbopol 940, Triethanolamine (TEA), and Tween 80 as formulation variables, with response parameters consisting of percent transmittance, pH, and viscosity, tested on formulation F1 containing EFJM. This actual design model was used to optimize the gel base formulation of the EFJM nano spray gel. Based on the analysis and optimization results using Design Expert 13, a total of nine recommended solutions were generated.

Physical Quality Test of EFJM Nano Spray Gel Preparation:
The results of the pH test analysis are shown in Figure 2.

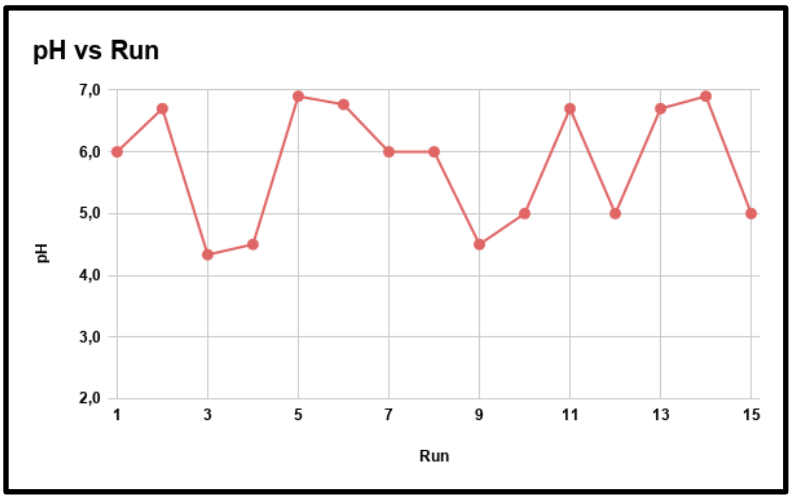


Figure 2. pH Value

The pH value of nano spray gel formulations plays an important role in both the effectiveness of preservatives and user comfort during topical application. In this study, the pH values of the formulations ranged from approximately 4.0 to 7.5, depending on the formulation run and replication, indicating a fairly wide variation across different compositions. This variation is likely influenced by the concentration of Carbopol 940 and the use of Triethanolamine (TEA) as a pH-adjusting agent. Carbopol is known to be acidic in nature (pH ±3), whereas TEA functions as a neutralizing agent, increasing the pH to form a stable gel matrix (22). Formulations with pH values below 4.0 may cause mild irritation to sensitive skin, while pH values above 7.0 can potentially disrupt the skin's acid mantle. The ideal pH range for topical preparations is typically between 4.5 and 6.5, aligning with the physiological pH of human skin (23).

Statistical analysis using Design Expert version 13 and the Simplex Lattice Design (SLD) method indicated that the linear model was the most suitable for predicting the pH response. This was supported by the Fit Summary and Sequential Model Sum of Squares, where the linear model demonstrated a significant p-value of 0.0006 ($p < 0.05$) and a non-significant lack of fit value of 0.4103 ($p > 0.05$), indicating the model had no systematic error and fit the experimental data well.

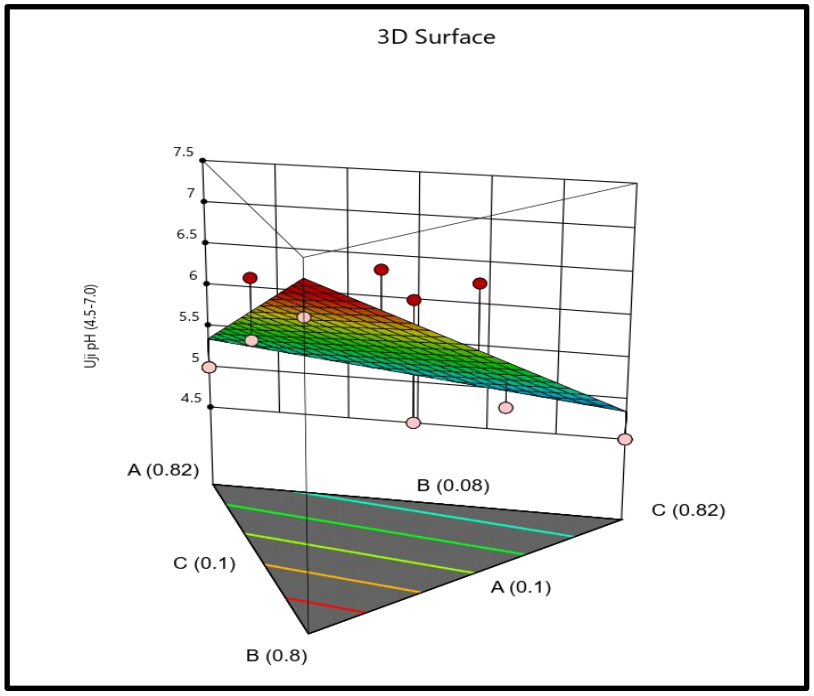


Figure 3. Coding Components of the Actual SLD Response pH Test

Figure 3 presents a contour plot of the pH response, represented by a color gradient ranging from blue (pH 4.5) to red (pH 6.9). The three vertices of the triangle correspond to the maximum concentrations of the three formulation components: A (Carbopol 940), B (TEA), and C (Tween 80). The concentrations of each component increase toward their respective corners. Red dots on the plot represent actual experimental data points. Further statistical analysis of the pH data using SPSS indicated that the dataset was not normally distributed ($p < 0.05$), necessitating the use of the non-parametric Kruskal–Wallis test. The results of the Kruskal–Wallis analysis showed no statistically significant difference between formulation groups ($p = 0.965$), suggesting that the various formulations maintained a consistent and safe pH range for skin application.

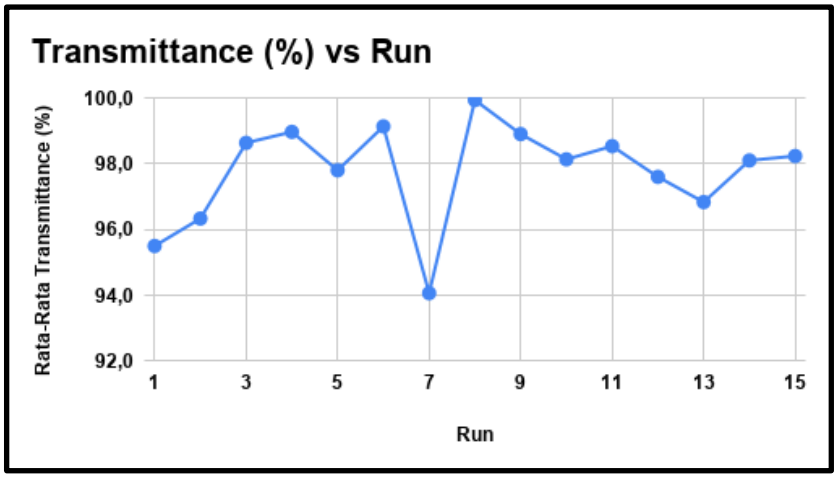


Figure 4. Transmittance Value

Based on the data presented in Figure 4, the formulations exhibited an average transmittance value of 97.8%, with a range between 94.1% and 99.9%. These results indicate that the nano spray gel formulations produced a clear and stable nanoemulsion system, characterized by a small droplet size and high optical clarity. According to the literature, a transmittance value exceeding 90% is categorized as a transparent nanoemulsion (13). The analysis of transmittance data using Design Expert 13 and the Simplex Lattice Design (SLD) method revealed that the quadratic model was statistically significant for this response. The ANOVA results showed a p-value of 0.018 ($p < 0.05$) and an F-value of 4.70, indicating that the interaction among formulation components had a significant effect on the clarity of the nano spray gel.

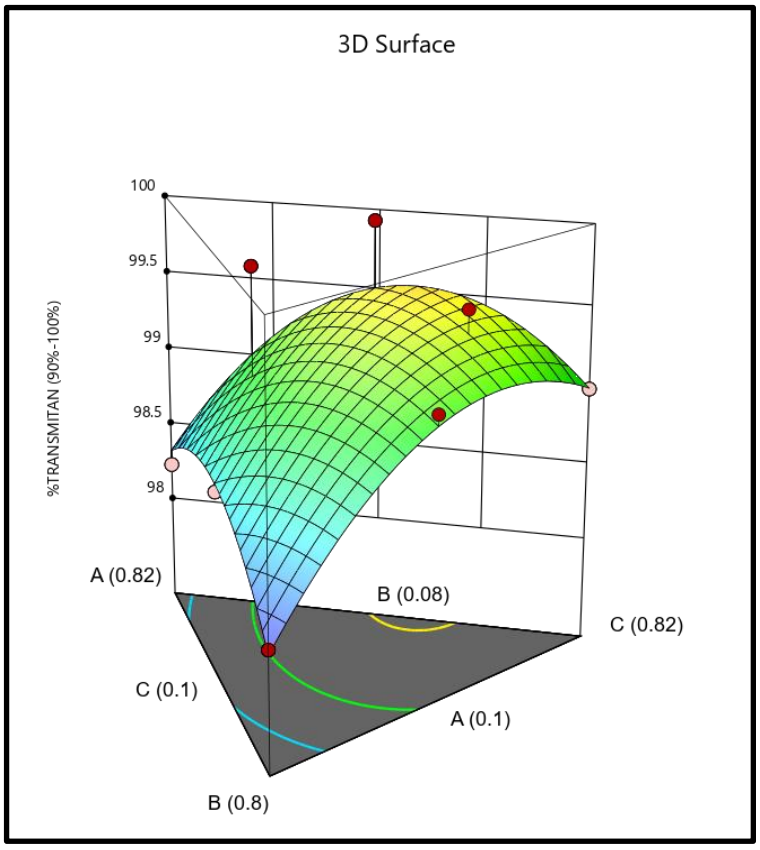


Figure 5. Coding Components Actual SLD Response % Transmittance

Figure 5 illustrates the distribution of % transmittance values, which fall within the 90% to 100% range, across three formulation components: Component A Carbopol 940 (%); Component B, Triethanolamine (TEA) (%); and Component C, Tween 80 (%). In both the contour plot and the three-dimensional surface plot, an increase in the proportion of Carbopol 940 (Component A) is associated with a higher percentage of transmittance. The % transmittance data were analyzed using SPSS, where the normality test revealed a p-value > 0.05, indicating that the data were normally distributed. Thus, parametric analysis using one-way ANOVA was deemed appropriate. Additionally, the homogeneity of variance was confirmed with a Levene's test p-value of 0.999, suggesting that all groups had equal variance, which satisfies the assumption required for ANOVA. The ANOVA results showed a p-value of 0.973, indicating that there were no statistically significant differences in %

transmittance among the formulation replicates. All replicates exhibited a high average transmittance of approximately 97.7%, demonstrating that the formulations consistently achieved excellent optical clarity.

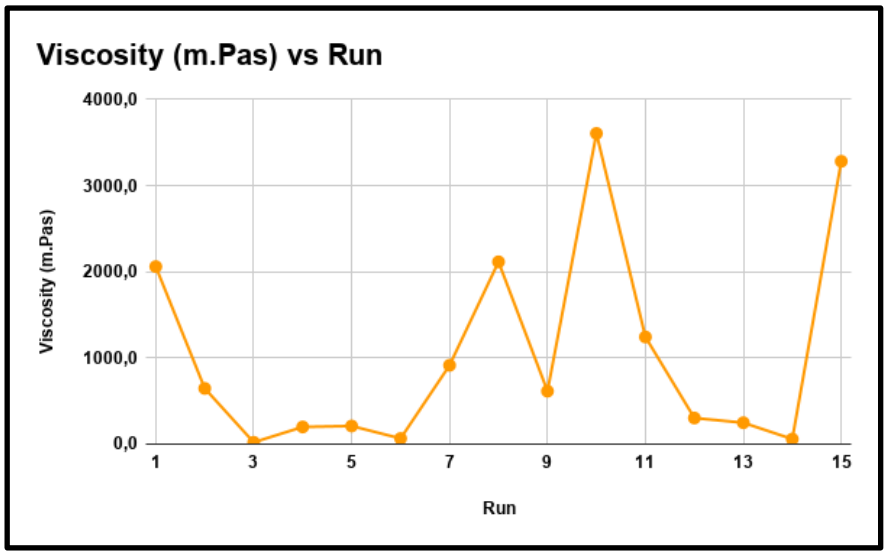


Figure 6. Viscosity Value

The viscosity test results demonstrated a relatively wide variation across formulations, with values ranging from 20 to 3600 mPa·s. This variation indicates that the inclusion of fermented red ginger extract tends to reduce the viscosity of the gel system. However, viscosity is also strongly influenced by the concentrations of Carbopol 940, Triethanolamine (TEA), and Tween 80 used in the formulation (24). In general, the optimal viscosity for nano spray gel formulations is considered to lie within the range of 500–2500 cP, as it balances application comfort with stability (25). Statistical analysis conducted using Design Expert 13 with the Simplex Lattice Design (SLD) method, revealed that the linear model was the most suitable for describing the viscosity response of the formulations. The Analysis of Variance (ANOVA) results also affirmed the model's significance, with a p-value <0.0001. At the same time, the Lack of Fit test yielded a non-significant result ($p = 0.6329$), indicating that the model adequately fits the experimental data without systematic error. Therefore, the model is deemed both statistically valid and reliable, and there is no necessity to increase model complexity. Further supporting evidence is found in the Fit Statistics, where the R^2 value of 0.8671 confirms that approximately 87% of the variability in viscosity is explained by the model.

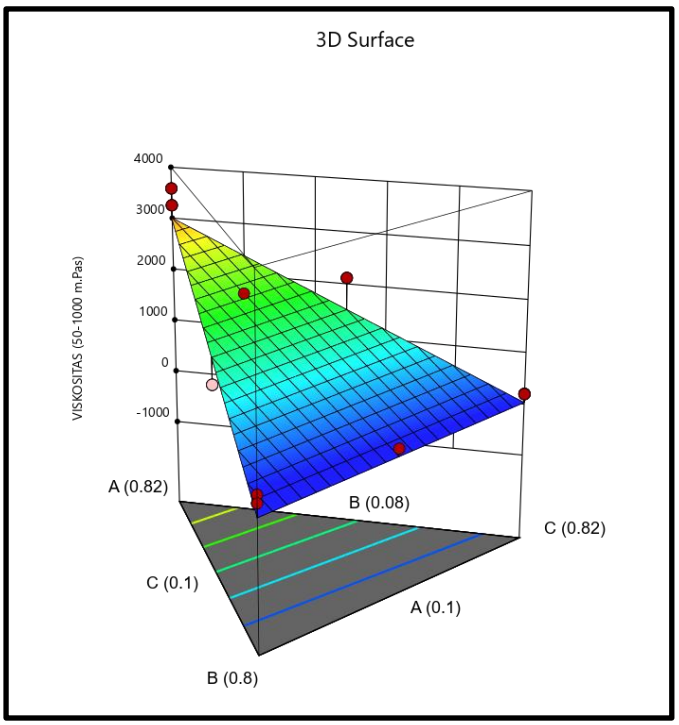


Figure 7. Actual SLD Response Coding Components of the Viscosity Test

Statistical analysis of the viscosity test data using SPSS revealed that the data were not normally distributed ($p < 0.05$); therefore, a non-parametric Kruskal–Wallis test was conducted. The results of the Kruskal–Wallis test showed no statistically significant differences between the formulation groups ($p = 0.991$), indicating that the formulations demonstrated good and consistent viscosity stability across the three replications. Stable viscosity is crucial for ensuring spray comfort and effective adhesion of the preparation to the skin surface.

The organoleptic evaluation of the formulation revealed that the spray gel exhibited a slightly viscous (sticky) liquid consistency, a clear yellow appearance, and a distinctive aromatic scent characteristic of red ginger and spices. Upon application, the formulation imparted a soft and cooling sensation on the skin. The organoleptic test was conducted to assess the physical characteristics of the nano spray gel, specifically focusing on appearance, color, consistency, and odor. According to established criteria, an ideal spray gel formulation should appear clear, homogeneous, free from bubbles or turbidity, and should possess an odor that corresponds to the added active ingredients (26).

The phase separation test was performed to assess the physical stability of nano spray gel formulations containing fermented red ginger extract when subjected to mechanical stress. A formulation is considered physically stable if it does not exhibit phase separation, sedimentation, or lump formation. In this study, all formulations from Run 1 to Run 15 were subjected to centrifugation testing to simulate physical force. The results showed no visible phase separation in any of the samples, indicating that the entire set of formulations possessed excellent physical stability (14).

The results of the homogeneity test for the nano spray gel formulations demonstrated that all samples exhibited excellent homogeneity. Microscopic observation further confirmed a uniform particle distribution, with consistent droplet size and no visible

impurities. This suggests that the formulation was capable of accommodating complex natural extracts without compromising its physical consistency and dispersion quality (27).

The spray pattern test was conducted to evaluate the uniformity of distribution of the nano spray gel when applied using a manual spray pump. Based on the observations, the tested formulation exhibited a relatively homogeneous spray pattern, characterized by an even circular distribution. According to standard evaluation criteria for spray gel formulations (26).

The results indicated that the nano spray gel formulation exhibited relatively low spreadability, ranging from 4.0 to 4.8 cm, but higher adhesion values, ranging from 40 to 55 seconds. The increase in adhesion may be attributed to the presence of fermentation-derived secondary metabolites, such as flavonoids and phenolic compounds, which are known to enhance viscosity and strengthen intermolecular interactions, thereby allowing the preparation to adhere longer to the skin surface (28).

The drying time test was conducted to evaluate the duration required for the nano spray gel formulation to completely dry on the skin surface after application. Formulations with lower concentrations of Carbopol were found to have faster drying times, approximately 2 to 4 minutes, which are considered optimal for specific applications such as wound care (29). Although drying times exceeding 5 minutes may slightly reduce user satisfaction, they remain acceptable if they contribute to improved bioavailability and penetration of the active ingredients (30).

The optimal formulation of Solution Number 1 yielded results for the % transmittance test, pH test, and viscosity, which are presented in Table 3.

Table 3. Test Results of % Transmittance, pH, and Viscosity of Optimal Formula

Parameter	I	II	III	Data
% Transmittance	99	99	99,2	99,1 ± 0,12
pH	5,9	6	6	6,0 ± 0,06
Viscosity (mPa.S)	540	536	537	537,7 ± 2,08

As presented in Table 3, the experimental data were further processed using the Simplex Lattice Design (SLD) approach in Design Expert version 13. The results of experimental validation demonstrated excellent concordance between the predicted values generated by the model and the observed experimental outcomes for the three primary response variables: % transmittance, pH, and viscosity. The bias ratio was found to be negligible, indicating no significant deviation between predicted and actual values. This suggests that the predictive model performed with a high degree of accuracy. These findings not only reinforce the reliability of the experimental design but also validate the robustness of the optimization model, thereby offering a solid foundation for potential scale-up and future production-level formulation development.

The Particle Size Analysis (PSA) test was performed to determine the particle size and polydispersity index (PI) of the nanodispersion formulation. Nanoparticle formulations typically have particle sizes ranging from 1 to 100 nm (31). A polydispersity index value approaching zero indicates a higher uniformity of droplet size within the formulation (32).

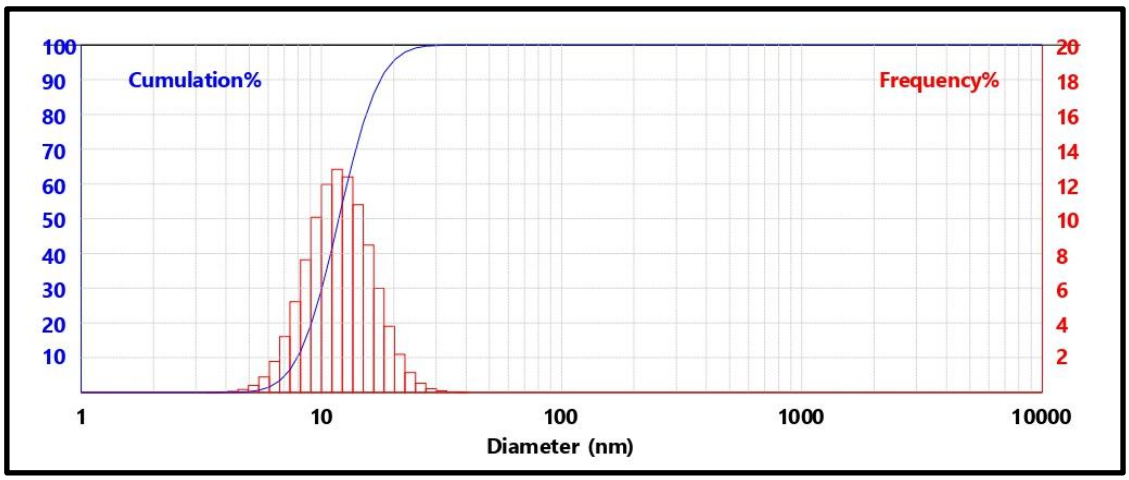


Figure 8. PSA Test Results

Based on the data presented in Figure 5, the Particle Size Analysis (PSA) of the nanodispersion formulation containing red ginger fermentation extract (EFJM) yielded a D90 value of 17.66 nm. This value indicates that 90% of the particles are smaller than 17.66 nm, classifying them within the nanoparticle range. The polydispersity index (PDI) of 0.1012 reflects a very narrow particle size distribution, indicating that the formulation is homogeneous and physically stable. Generally, a PDI value below 0.3 indicates a highly uniform nanoparticle system, with lower PDI values being associated with greater physical stability and a reduced tendency for particle aggregation. Moreover, particle sizes below 100 nm are associated with enhanced penetration (33). The PSA results demonstrate that the employed formulation method, sonication process, and the use of surfactants (Tween 80) successfully produced nanodispersion formulations of EFJM with stable and homogeneous nanoparticle sizes.

In the inflammatory process in the feet of mice after being given 1% carrageenan injected intraplantarly (into the sole of the mouse's foot). Treatment was given, and the volume of the mice's feet was measured every 30 minutes, 60 minutes, and 90 minutes. The average AUC and % anti-inflammatory power values are presented in Table 4.

Table 4. Average AUC (Area Under Curve) and Percentage of anti-inflammatory activity (%AIA)

Parameter	Average AUC (mL.hour)			%AIA (Anti-inflammatory activity)		
	1/2 hour	1 hour	1 1/2 hour	1/2 hour	1 hour	1 1/2 hour
Control -	0,0900	0,0815	0,0750	0,0000	0,0000	0,0000
Control +	0,0645	0,0330	0,0180	28,3333	59,5092	76,0000
EJM	0,0790	0,0340	0,0065	12,2222	58,2822	91,3333
EFJM	0,0630	0,0305	0,0115	30,0000	62,5767	84,6667
Nano spray EFJM	0,0905	0,0405	0,0145	-0,5556	50,3067	80,6667

The AUC (Area Under Curve) indicates the average area under the curve, which represents the relationship between the average paw volume of mice over time. A decreasing AUC value indicates that the inflammation that occurred was inhibited after treatment.

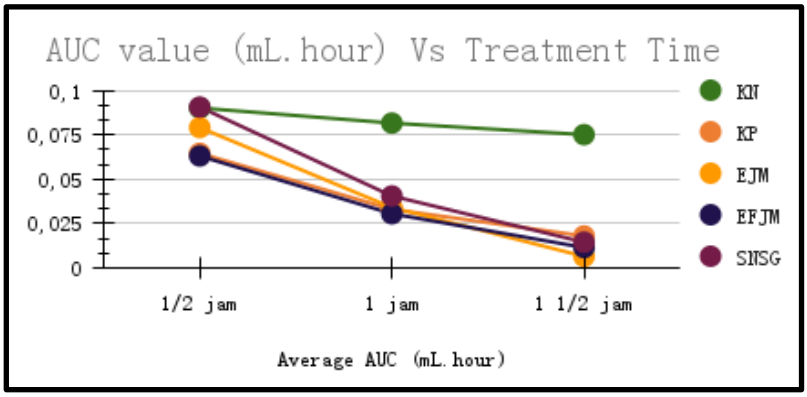


Figure 9. AUC Value (mL.hour) and Treatment Time

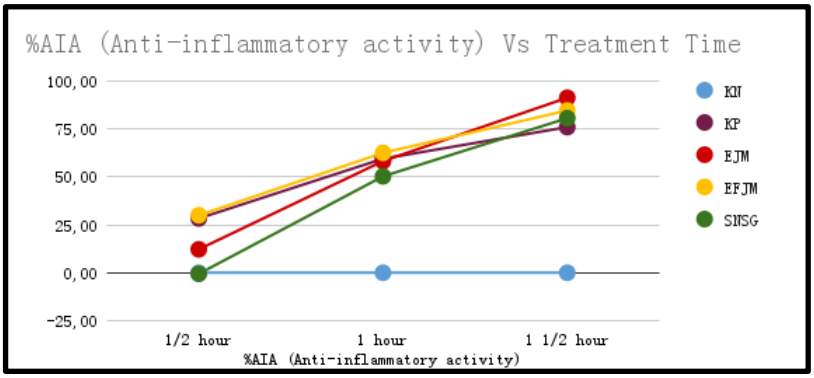


Figure 10. % AIA (Anti-inflammatory Activity Vs Treatment Time

A lower AUC value corresponds to a higher percentage of anti-inflammatory activity (%AIA) (20). The nano spray gel containing fermented red ginger extract demonstrated notable anti-inflammatory activity, with an AUC value of 0.0145 and an AIA of 80.67% after 1.5 hours of treatment, indicating effective inhibition of inflammation. The nano spray gel containing fermented red ginger extract investigated in this study was a preliminary formulation prior to the optimization of the gel base using a simplex lattice design. Therefore, in vivo evaluation of the optimized formulation was not performed and should be considered in future studies.

Normality tests conducted using SPSS for the positive control, fermentation extract, thick extract, and preparation groups yielded p-values greater than 0.05, indicating that the data were normally distributed. In contrast, the negative control group showed a p-value below 0.05, likely due to the lack of treatment, which led to increased variability and a non-normal data distribution. Therefore, the data were analyzed using the Kruskal-Wallis test. The results for the KP, EJM, EFJM, and SNSG groups revealed a Kruskal-Wallis statistic with a p-value <0.001, indicating statistically significant differences among the groups.

CLINICAL IMPLICATION

The clinical implication of this study lies in the development of a potential anti-inflammatory nanospray gel containing red ginger fermented extract through formulation optimization.

LIMITATIONS

A limitation of this study is that the formulation design was evaluated only based on transmittance, pH, and viscosity parameters. Therefore, it is necessary to include additional parameters, such as encapsulation efficiency (% EE), in future assessments.

CONCLUSIONS

The optimal nano spray gel formulation consisted of Carbopol 940 (0.243), triethanolamine (TEA) (0.166), and Tween 80 (0.591), yielding a desirability value of 0.948. The optimized formulation exhibited favorable physicochemical characteristics, including a pH of 6.0 ± 0.06, transmittance of 99.1 ± 0.12%, and viscosity of 537.7 ± 2.08 mPa·s. Particle size analysis revealed a mean particle diameter of 14.35 nm, confirming the successful formation of a nanodispersion system. These findings demonstrate that the Simplex Lattice Design (SLD) approach is an effective strategy for optimizing the formulation of nano spray gel containing fermented red ginger extract to achieve the desired physicochemical properties. In the in vivo anti-inflammatory study, statistically significant differences were observed among the red ginger extract (EJM), fermented red ginger extract (EFJM), and pre-optimization nano spray gel (NSG) groups ($p < 0.001$). The nano spray gel containing fermented red ginger extract exhibited the greatest anti-inflammatory activity, with an area under the curve (AUC) value of 0.0145 and a percentage of anti-inflammatory activity (%AIA) of 80.67% at 1.5 h after treatment, indicating effective suppression of inflammation. Despite these promising findings, several limitations should be acknowledged. The encapsulation efficiency (%EE) of the optimized formulation was not determined, and the optimized nano spray gel generated using the SLD approach was not evaluated in vivo. Therefore, future studies should determine the encapsulation efficiency and investigate the in vivo therapeutic efficacy of the optimized formulation to further validate its potential for development as a natural topical anti-inflammatory pharmaceutical product.

CONFLICT OF INTEREST

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

AUTOR CONTRIBUTIONS

N.W.R.K.D. designed and conducted the research, analyzed and interpreted the data, and drafted the manuscript. M.D.S.S. contributed to the research design, supervised the research process, and reviewed the manuscript. N.K.K.J.P.S.W. participated in the research design and execution, drafted and critically reviewed the manuscript, and provided supervision throughout the study. All authors contributed to the research implementation and approved the final manuscript version.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors used ChatGPT (OpenAI, version GPT-5.1) 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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