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Research Article

Nanoemulgel Formulation of *Eleutherine palmifolia* Onion Bulb Extract and Its Effectiveness in Wound Healing

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ABSTRACT

Eleutherine palmifolia onion, an indigenous medicinal plant from Borneo, is empirically applied to treat wound healing. The objective of this study is to formulate its extract and assess its *in vivo* wound healing activity. *Eleutherine palmifolia* onion was extracted with a 96% ethanol solvent. The extract of the Dayak onion bulb was then formulated into a nanoemulsion by applying the DOE method of 2-level factorial design. Four nanoemulsion formulations were further analyzed to obtain the optimal formulation. The particle size analysis and % transmittance was performed using a particle size analyzer and an ultraviolet-visible spectrophotometer. Furthermore, the data were analyzed using the Minitab 19 application with the DOE factorial design method. The three nanoemulgel formulations containing 2.5, 5.0, and 7.5% extract were further investigated for wound healing efficacy in rats. The duration of wound closure was analyzed using paired t-tests. The best nanoemulsion formulation was made with 22.5 mL of Tween 80 and 2 mL of isopropyl myristate oil. This produced particles that were 12.13 nm in size and a 99.12% transmittance rate. Wound length measurements were taken daily for 15 days. The statistical test results of p<0.05 indicate that the nanoemulgel of the extract has significant activity in wound healing. The optimal nanoemulsion formula is formula A, and the effective dose of Dayak onion extract nanoemulgel is 5% and 7.5% concentrations, with a wound healing time of 13 days.

INTRODUCTION

New drug delivery systems are rapidly evolving as a result of successes in various disciplines such as biomedical engineering, biotechnology, and nanotechnology. In pharmaceuticals, cutting-edge nanotechnology is used to prepare drugs in the form of nanoparticles to be effectively absorbed, thereby providing a pharmacological response with minimal side effects through controlled drug release methods (Qushawy and Nasr, 2020). In recent years, nanotechnology has been introduced as a relatively new field of science and technology dealing with nanometer-sized materials for medical purposes. The application of nanotechnology for medical purposes has been called nanomedicine and is described as the application of nanomaterials for the diagnosis, monitoring, control, prevention, and treatment of disease (Subair and Mohanan, 2022). It is an important engineering field in physics, chemistry, and biology, and various types of nanotechnology are developing very rapidly, namely nanomedicine, nanoemulsions, and nanoparticles. Nanoparticles are one of the technologies that can elevate the effectiveness of drug delivery (Christania *et al.*, 2020; Kumowal *et al.*, 2019). Nanotechnology has particles with a size of less than 1000 nm. With a smaller particle size, nanoparticles have a larger surface area and better physical and chemical properties (Fitri *et al.*, 2020; Setiawan *et al.*, 2020).

The application of plants as traditional medicine by the Indonesian people as an alternative source of modern medicine has increased, in addition to low prices, easy availability, and fewer side effects compared to chemical-based drugs (Sari *et al.*, 2017). One method of application is by applying grated *Eleutherine palmifolia*, known as Dayak onion, to the injured area or by drinking boiled water (Novaryatiin *et al.*, 2018). Empirically, this plant is well-known to have anti-inflammatory, antimicrobial, anti-breast cancer, stroke prevention, and cholesterol-lowering effects (Wijayanti *et al.*, 2018; Wigati and Rahardian, 2018). *Eleutherine palmifolia* bulbs contain naphthoquinone compounds, flavonoids, polyphenols (Muti'ah *et al.*, 2020; Sari *et al.*, 2017), alkaloids, glycosides, phenolics, steroids, tannins, and saponins (Seja *et al.*, 2018; Kumalasari *et al.*, 2020). Active ingredients used for wound healing are tannins, saponins, and flavonoids (Calsum *et al.*, 2018; Lallo *et al.*, 2020). Vitexin is one of the flavonoid compounds that can act as a wound healer through anti-inflammatory mechanisms. Vitexin has poor water solubility, resulting in poor bioavailability and low absorption (Abdulai *et al.*, 2021; Peng *et al.*, 2021). The poor aqueous solubility of vitexin limits its dissolution, which greatly affects the bioavailability of the drug (Zu *et al.*, 2012).

A nanoemulgel system is the synergetic combination of nanoemulsion and the gelling system in the form of a semisolid preparation (Nastiti *et al.*, 2023). The oil selection used in nanoemulsion formulation is an important factor that the drug will incorporate as a droplet in the oil phase dispersed in the aqueous phase. The oil phase component will help to achieve maximum drug loading in the nanoemulsion system (Dwiastuti *et al.*, 2023). Nanoemulsion is an oil and water dispersion system with nano-sized globules that have good stability with the addition of surfactants and cosurfactants (Priani *et al.*, 2021). Nanoemulsion is a part of nanotechnology that has been widely developed in nanomedicine and nanodermatology to improve the performance of medical materials, especially those that are difficult to dissolve in water or vice versa (Wulansari *et al.*, 2019). Nanoemulsions have several advantages, namely that their very small size, ranging from 50 to 1000 nm, facilitates the absorption rate of lipophilic and hydrophilic drugs into the body (Soegiharto *et al.*, 2022).

Indonesian people use many natural ingredients for wound care, and *Eleutherine palmifolia* onion extract is one of the plants that is believed to be able to effectively treat wounds. The content of active compounds such as flavonoids, tannins, and saponins is a group of compounds that have the potential to be applied in the fabrication process of wound-healing drugs. Natural wound care preparations are combined with nanoparticle technology to make wound healing preparations. *Eleutherine palmifolia* onion extract nanoemulgel preparation needs further research to determine its activity in wound healing. This needs to be done in order to develop anti-wound preparations with natural ingredients.

METHODS

Materials

Borneo onion bulb. Re-distilled water, tween 80, and isopropyl myristate were purchased from Jianling, along with propylene glycol (Brataco) and ethanol (Supelco).

Instrumentation

Hot plate (Thermo), particle size analyzer (Horiba), sonicator, spectrophotometer (Shimadzu), rotary evaporator, analytical balance (OHAUS), water bath, shaker, and drying oven.

Preparation of Dayak Onion Bulb Extract

Drying of simplicia was done by aeration or not being exposed to direct sunlight for 14 days at room temperature and then using an oven for 48 hours at a temperature of 45 °C until dry *Eleutherine palmifolia extract* was prepared by the maceration method. First, 500 g of its powder was put into each Erlenmeyer and soaked with 96% ethanol solvent, as much as 5 L, and then stirred for 24 hours using a shaker. This procedure was repeated three times to obtain the optimum mixture. Stirring with a shaker for 24 hours aims to maximize the contact of the solvent with the sample so that it can facilitate filtering. Furthermore, the extract was filtered to obtain a red extract containing *Eleutherine palmifolia* extract. The filtrate was then concentrated using a vacuum rotary evaporator at a temperature of 45 °C and then dried in an oven at a temperature of 45 °C until a fixed weight is obtained.

The experiment commenced with a 2-level factorial design, with the selected factors being the quantities of isopropyl myristate and Tween. We focused on measuring two critical responses: globule diameter size and % transmittance. These responses were anticipated to align with

Table 1. Optimization factors and levels

Factor	Low value (-)	High value (+)
Tween 80 (A)	10 mL	22.5 mL
Isopropyl myristate (B)	2 mL	4 mL

Table 2. Particle Size Analyzer (PSA) optimization design and % transmittance

Treatment	A	B
F1	10 mL (-)	2 mL (-)
FA	22.5 mL (+)	2 mL (-)
FB	10 mL (-)	4 mL (+)
FAB	22.5 mL (+)	4 mL (+)

Table 3. 2-Level Factorial Design of Dayak Onion Extract Nanoemulsion Formulation

Material	F1	FA	FB	FAB
Dayak onion extract	2.5 g	2.5 g	2.5 g	2.5 g
Isopropyl myristate	2 mL	2 mL	4 mL	4 mL
Tween 80	10 mL	22.5 mL	10 mL	22.5 mL
Propylene glycol	22.5 mL	22.5 mL	22.5 mL	22.5 mL
Water Pro Injection	45 mL	45 mL	45 mL	45 mL
Ethanol 96%	5 mL	5 mL	5 mL	5 mL

the desired target values for globule size and % transmittance in the nanoemulsion. For each factor, the lowest and highest values obtained were recorded from prior experiments, signifying that the nanoemulsion system had already been formed within the predetermined range of values. Subsequently, these recorded values underwent statistical analysis using the design of experiments in Minitab version 19 (Tables 1 and 2).

Nanoemulsion preparation

It was an oil-in-water (O/W) nanoemulsion, and the oil phase, surfactant, and co-surfactant were made up of isopropyl myristate, Tween 80, and propylene glycol. The formulation was optimized by varying the amount of oil and surfactant. The composition of oil, surfactant, co-surfactant, and the stirring process (time and speed) are factors that play a role in nanoemulsion formulation optimization. The determination of these factors was done through a 2-level factorial design of experiments and continued with the Box-Behnken method using Minitab 19 software.

The first stage of the experimental design is a preliminary test to identify the factors that are significant for the outcome response. One method used is a 2-level factorial design (2n), which means that n factors consist of 2 levels, namely low (-) and high (+). The low (-) level is the lower limit of the level, and the high (+) level is the upper limit of the level in the range of factor levels. The second step is to evaluate the factors and interactions that occur using Full Factorial Design, which is also one of the experimental design techniques for mathematical and statistical modelling based on polynomial equations from experimental data. The response of various factors is in the form of particle size and the percentage transmittance produced by each design formula. The expected target particle size is less than 100 nm, and the transmittance is 90–100% (Widyastuti and Saryanti, 2023).

The factorial design then produces a normal plot analysis, which identifies the factors that have a significant effect on the response. Further mathematical modelling uses the Box Behnken Design (BBD) based on the results of factor screening in the 2-level factorial design. The nanoemulsion formula is then created based on the test responses generated after analysis using Minitab 19 software. The BBD is a method that can be used to optimize a formulation. The Box-Behnken analysis then produces a polynomial equation for the size and transmittance responses that gives the optimal composition of the expected nanoemulsion formula.

Materials included *Eleutherine palmifolia* extract (active ingredient), isopropyl myristate (oil), Tween 80 (surfactant), and propylene glycol (co-surfactant) water phase (re-distilled water) The nanoemulsion was prepared by first dissolving the Dayak onion bulb extract in 5 mL of 96% ethanol using a stirrer until dissolved. The dissolved extract was then added drop by drop to propylene glycol while stirring with a stirrer at a speed of 10 for 5 minutes at a temperature of 45 ºC. Tween 80 was then added drop by drop while still stirring at speed 10 for 5 minutes at 45 ºC to homogenize the mixture. Isopropyl myristate (oil) was then added dropwise to the mixture while stirring at a speed of 10 for 5 minutes at 45 ºC. The aqueous phase was added dropwise while maintaining rotation and temperature. The resulting emulsion was then sonicated at 40°C for 20 minutes and then processed for particle size and transmittance testing.

Nanoemulgel preparation

The nanoemulgel was prepared by first developing Carbopol 940 in hot re-distilled water (20 mL). The expanded carbopol was stirred until homogeneous. Subsequently, triethanolamine was added and stirred until homogeneous. Furthermore, the nanoemulsion was gradually added while stirring until a homogeneous gel mass was formed.

Wound Healing Activity

Before testing how well a nanoemulgel with *Eleutherine palmifolia* extract healed biopsy wounds in rats, we received permission to conduct the study from the Ethics Committee of the Integrated Research and Testing Laboratory at Universitas Gadjah Mada in Yogyakarta, Indonesia. The study was conducted in compliance with the approved ethical clearance and was assigned the ethical clearance certificate number 00010/04/LPPT/IV/2023. Wistar rats were sourced from the Centre for Food and Nutrition Studies at Universitas Gadjah Mada. The laboratory maintained controlled room conditions, including a temperature range of 20–25 °C, humidity at 70%, a 12-hour light and 12-hour dark cycle, stainless steel individual maintenance cages, daily bedding cleaning, and biosafety laboratory level 1 facilities. The male and female Wistar rats, weighing between 150 and 200 grams, were divided into six treatment groups. Group I received no treatment; Group II served as the negative control and received gel base without extract; Group III was the positive control and received Cutimed gel; and Group IV, Group V, and Group VI were treated with nanoemulgels containing extract at concentrations of 2.5%, 5%, and 7.5%, respectively. The parameter we observed in this study was wound closure on the backs of male and female white rats. The wound closure was observed and measured every day from day 0 until day 15.

RESULTS AND DISCUSSION

The amount of isopropyl myristate used in the formula varied between 2-4 mL, while the surfactant and Tween 80 ranged from 10–22.5 mL, as indicated in Table 2. Based on the values of each factor from Table 2, we derived four variations of the 2-level factorial design, which are presented in Table 3. The data from the significant factor screening were subjected to significance testing at a 95% confidence level ($\alpha = 0.05$). The outcomes are visually represented in the normal plot graph in Figure 2.

The nanoemulsion preparations listed in Table 4 exhibit a transparent appearance, which is evident from the transmittance value of approximately 100%. This transparency indicates that the nanoparticle system formed in the preparation allows almost all incident light rays to pass through during spectrophotometer testing. The % transmittance results reveal that replication formulations 1-3 fall within the % transmittance range of 98.00% to 99.57%. These findings indicate that all formulations successfully meet the transmittance criteria. A well-formulated nanoemulsion typically exhibits a transmittance of 90–100%, signifying that it possesses small particle sizes (Widyastuti and Saryanti, 2023).

Table 4. Results Particle Size and % transmittance (n=3, expressed as mean ± SD)

Treatment	Particle Size (nm)	Transmittance (%)
F1	98.88 ± 0.65	23.77 ± 0.57
FA	99.13 ± 0.40	12.13 ± 0.12
FB	99.08 ± 0.32	106.5 ± 1.67
FAB	99.42 ± 0.17	14.07 ± 0.32

Regarding the Particle Size Analysis (PSA) test, the results obtained fall within the range of 12.0-108.6 nm. These results confirm that the particle size values of the samples qualify as nanoparticle size. The PSA operates on the principle of laser light scattering on sample particles, which are rapidly detected by a photon detector at a specific angle to determine particle sizes in a sample or preparation. Nanoemulsion preparations typically feature average droplet sizes ranging from 1–500 nm (Zulfa, 2019).

The normal plot graph illustrates the significance of the factors affecting the response. In Figure 1, the particle size responses of the two factors that have a significant effect on particle diameter size are Formula A, Formula B, and Formula AB, where the result was p <0.05, Tween 80, as a surfactant in nanoemulsions, functions to emulsify oil by adsorbing on the surface of oil globules to form a monolayer and reduce the interfacial tension between oil and water. Changes in the amount of surfactant will affect the physical properties of the emulsion, one of

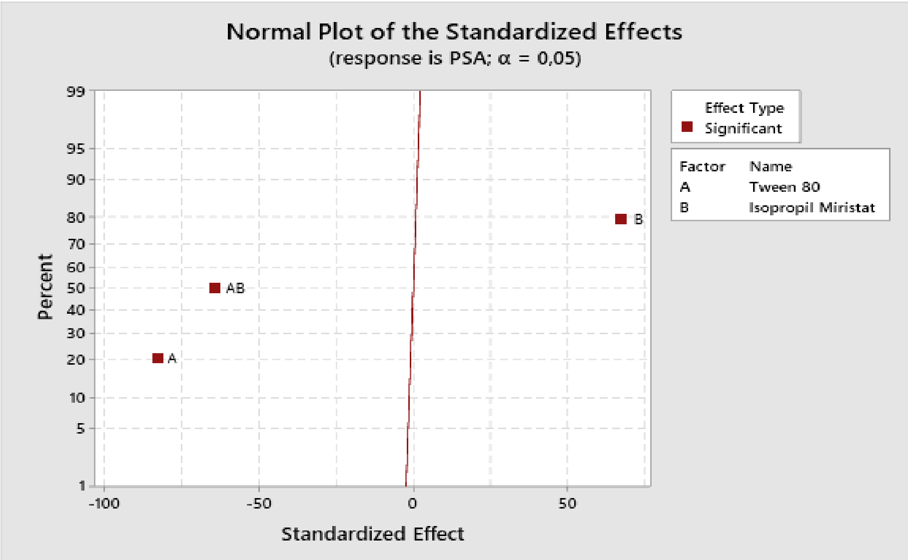

Figure 1. Normal plot graph illustrating the effect of factors on the response of globule diameter size Factor A = amount of Tween 80, B = isopropyl myristate, and AB = ratio of Tween 80 - isopropyl myristate. All factors were significant: A, B, and AB.

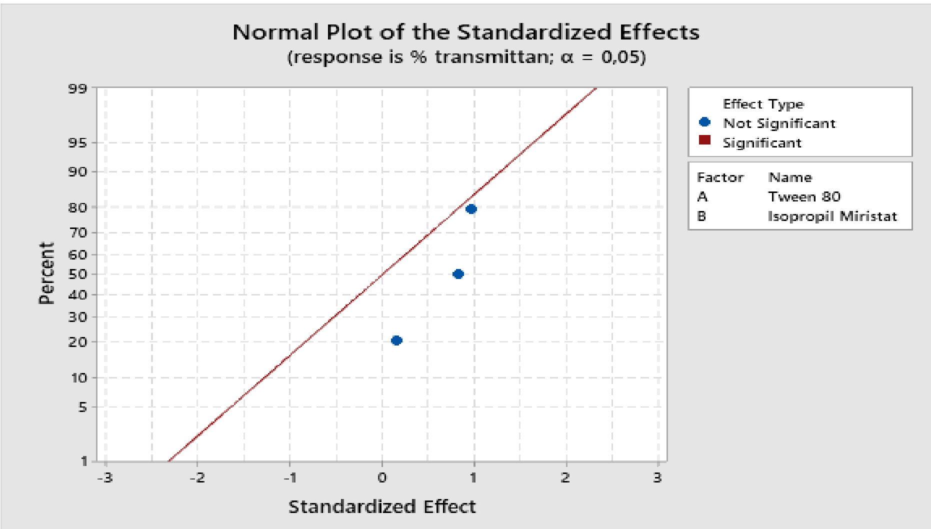

Figure 2. Normal plot graph illustrating the effect of factors on the response of globule diameter size Factor A=amount of Tween 80, B=isopropyl myristate, and AB=ratio of Tween 80 – isopropyl myristate. All Factors were significant: A, B, and AB.

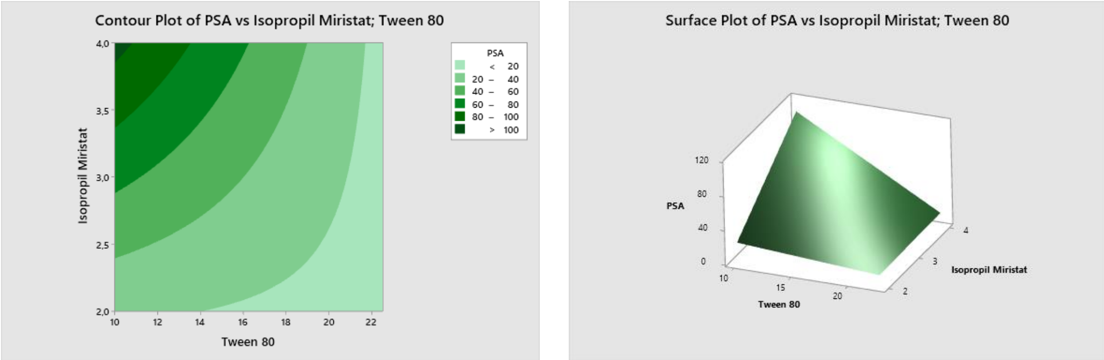

Figure 3. Contour plot of 2/3-dimensional factorial design Box-Behnken Tween 80 and isopropyl myristate nanoemulsion on particle size response

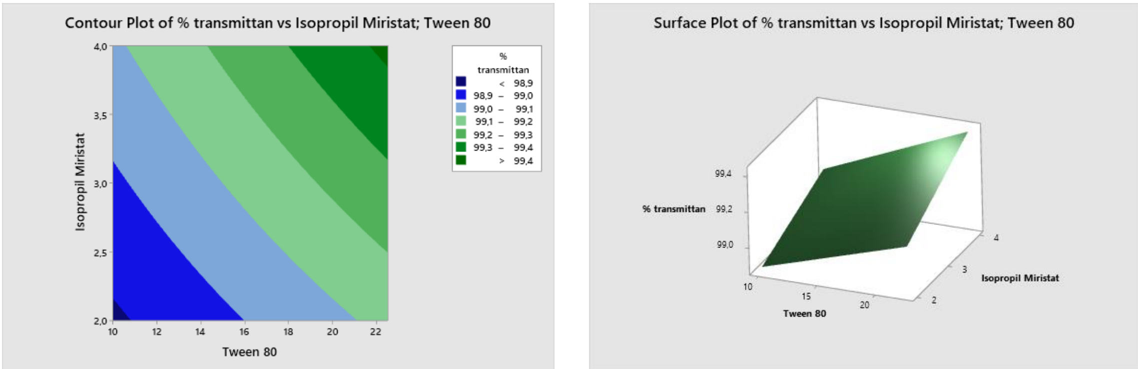

Figure 4. Contour plot of a 2/3-dimensional factorial design Box-Behnken Tween 80 and isopropyl myristate nanoemulsion on transmittance response.

which is the size of the globule diameter (Nursal *et al.*, 2019). IPM is a low-molecular-weight, non-triglyceride oil with characteristics of high polarity, low interfacial tension, and low viscosity. Oils with these characteristics are advantageous in the preparation of nanoemulsions because they are easier to produce in nanosize compared to mineral oils and vegetable oils, which have large molecular sizes (Prihantini *et al.*, 2018).

A result with $p > 0.05$ indicates the % transmittance responses of the two factors, specifically Formula A, Formula B, and Formula AB, did not exhibit a significant effect on % transmittance (Figure 3). The clarity of nanoemulsions is often quantified using transmittance. Tween 80, being a surfactant, primarily functions to reduce the surface tension between two phases. In this study, it was observed that Tween 80 did not have a statistically significant impact on the clarity of the nanoemulsion (Putri *et al.*, 2020).

Isopropyl myristate, on the other hand, is among the penetration enhancers used in topical preparations. It operates by reconstituting fats, thereby enhancing permeability. In this study, it was determined that isopropyl myristate did not exert a statistically significant influence on the clarity of the nanoemulsion (Nurleni, Erviana, & Firdiawan, 2022).

Nanoemulsion with a size < 20 nm can be produced by the Tween formula as much as 14–22.5 mL, and isopropyl myristate 2–3.9 mL nanoemulsion with a transmittance> 99.4% can be produced by the Tween 80 formula as much as 21–22.5 mL and isopropyl myristate 3.8–4 mL (Figure 4). From the two mathematical models, the optimization response was determined to obtain the optimal formula according to the desired target with the Box-Behnken test, as shown in Figure 5. Based on the values of the first partial derivatives, Tween 80 (22.5 mL) and isopropyl myristate (2 mL), the mean response of the bead size was 12.13 nm, and the % transmittance was 99.12%.

Particle size testing is a crucial parameter for nanoemulsion preparations because it ensures the particle size falls within the desired range. This nanoparticle size plays a crucial role in enhancing the penetration of the preparation into the skin.

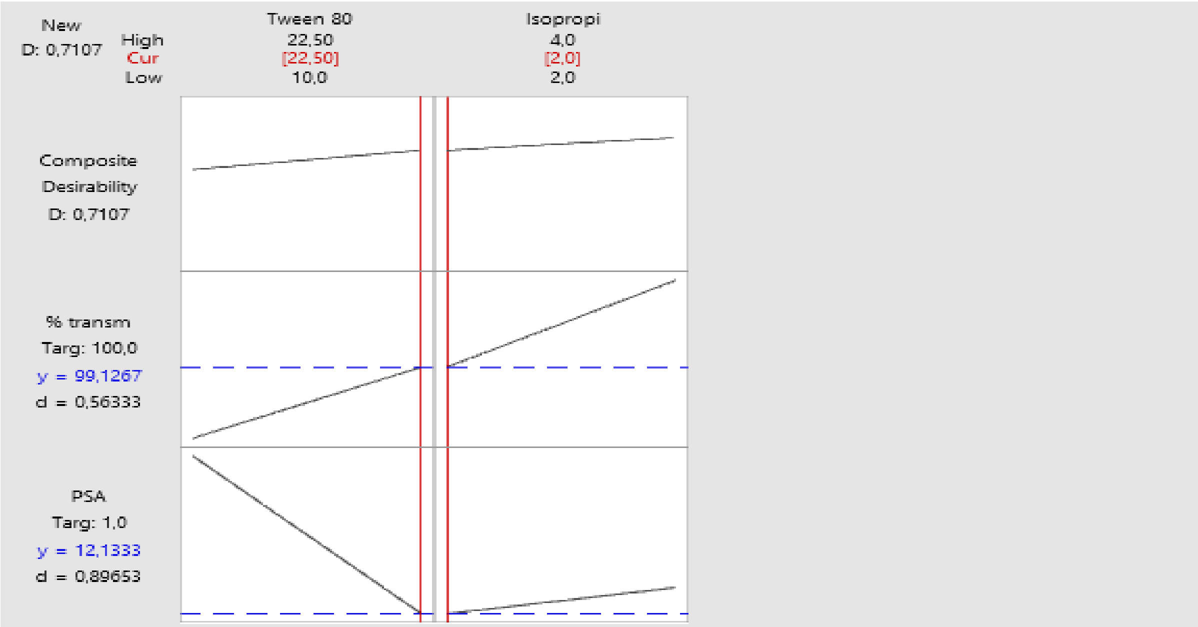

Figure 5. Graph of nanoemulsion formulation optimization results based on Box Behnken factorial design.

Table 5. Wound length observation results

Group	Wound Length Day - (mm)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
I	4	2.33 ± 0.26	1.63 ± 0.29	2.73 ± 0.21	2.57 ± 0.59	1.97 ± 0.21	2.10 ± 0.22	2.23 ± 0.40	1.70 ± 0.45	2.50 ± 0.36	2.13 ± 0.25	2.47 ± 0.41	1.30 ± 0.29	1.40 ± 0.22	1.07 ± 0.25	0.67 ± 0.33
II	4	2.43 ± 0.57	1.63 ± 0.34	2.87 ± 0.37	2.63 ± 0.09	2.37 ± 0.52	2.67 ± 0.17	2.33 ± 0.33	2.03 ± 0.54	1.93 ± 0.39	1.97 ± 0.33	2.40 ± 0.73	1.50 ± 0.36	1.43 ± 0.47	1.27 ± 0.49	1.43 ± 0.39
III	4	2.07 ± 0.17	1.83 ± 0.21	2.03 ± 0.26	2.50 ± 0.36	2.60 ± 0.33	2.63 ± 0.58	2.13 ± 0.34	2.27 ± 0.83	1.53 ± 0.05	1.47 ± 0.25	2.17 ± 0.21	1.43 ± 0.25	1.07 ± 0.57	0.83 ± 0.49	0.50 ± 0.71
IV	4	2.13 ± 0.26	1.83 ± 0.33	2.60 ± 0.43	2.07 ± 0.12	2.10 ± 0.42	2.17 ± 0.42	2.60 ± 0.70	2.70 ± 0.64	1.43 ± 0.21	1.53 ± 0.40	2.03 ± 0.29	1.17 ± 0.49	0.80 ± 0.78	0.57 ± 0.80	0.50 ± 0.71
V	4	2.43 ± 0.12	1.90 ± 0.29	2.80 ± 0.45	2.53 ± 0.17	2.80 ± 0.29	2.33 ± 0.40	1.70 ± 0.59	2.07 ± 1.05	0.77 ± 0.33	0.70 ± 0.16	0.60 ± 0.24	0.40 ± 0.16	0.23 ± 0.26	0.10 ± 0.14	0.07 ± 0.09
VI	4	2.60 ± 0.22	1.83 ± 0.21	2.33 ± 0.09	2.83 ± 0.05	2.63 ± 0.21	2.80 ± 0.64	2.53 ± 0.65	2.27 ± 1.03	1.17 ± 0.38	1.10 ± 0.29	0.97 ± 0.59	0.63 ± 0.21	0.23 ± 0.26	0.13 ± 0.19	0.10 ± 0.14

Data was expressed as mean ± standard deviation (SD) which n = 3.

Additionally, the polydispersity index (PDI) value holds significance since it provides insight into the particle distribution within the formulation. Another critical parameter to consider is the zeta potential value, which characterizes the electrical properties of the particles. The zeta potential holds substantial influence over the stability of the preparation because it directly impacts the attractive interactions between particles. Therefore, a higher zeta potential value can effectively prevent flocculation, contributing to the formulation's stability.

Based on data in Table 5, it is clear that the nanoemulgel with *Eleutherine palmifolia* extract heals biopsy wounds faster than both the negative control (gel base without extract) in group 2 and the positive control (Cutimed gel) in group 3. This better ability to heal wounds is due to bioactive compounds found in *Eleutherine palmifolia* extract, mainly the large number of flavonoid compounds (Febrinda *et al*., 2013). Flavonoid compounds possess noteworthy anti-inflammatory properties, with their mechanism of action cantered on inhibiting the enzyme cyclooxygenase (COX2). COX2 plays a pivotal role in the production of prostaglandins, which serve as mediators of inflammation. By suppressing COX2, the production of prostaglandins is effectively curtailed, thus halting the inflammatory process and expediting the wound healing process (Singh *et al*., 2022). The wound healing process has four phases, namely, the hemostatic, inflammatory, proliferation, and maturation phases.

The first hemostatic phase is the initial response that involves blood vessel reactions, specifically vasoconstriction and hemostasis, typically occurring 5–10 minutes after injury. During this phase, there is an increase in platelet adhesion. These platelets secrete factors that stimulate blood clot formation. Platelets then aggregate along the endothelium of blood vessels, leading to the conversion of fibrinogen into fibrin monomers, which collectively form a blood clot, preventing blood vessel leakage (Khairiah and Kusuma, 2021). Notably, on day 1, there is no significant change in wound healing since test animals are still undergoing vasodilation and blood flow around the wound remains absent.

Second is the inflammatory phase, which is characterized by vasoconstriction and platelet aggregation, which promote blood clotting. Subsequently, vasodilation and phagocytosis occur, initiating inflammation within the incision wound. The flavonoid content in the nanoemulsion derived from Dayak onion bulb extract exhibits effective anti-inflammatory properties. Flavonoids inhibit the release of arachidonic acid and lysosomal enzymes from cell membranes by blocking various pathways, including the cyclooxygenase, lipoxygenase, and phospholipase A2 pathways. This inhibition results in reduced arachidonic acid availability for the cyclooxygenase and lipoxygenase pathways, consequently decreasing prostaglandin and leukotriene production (Pariyana *et al*., 2018). The reduced prostaglandin production may abbreviate the inflammatory response, accelerating both healing and collagen formation. In treatment groups 1 and 2, characterized by a lack of treatment and the use of emulgel bases without anti-inflammatory drugs, the inflammatory phase persisted longer compared to the other four groups.

In the third proliferation phase, within treatment groups 4, 5, and 6, significant differences in wound healing percentages are observed, primarily attributed to higher extract concentrations in the nanoemulgel formulations. During this phase, flavonoids effectively stimulate macrophages to produce growth factors and cytokines, expediting the proliferation phase and wound healing process. Fibroblasts migrate to the wound area during this phase, gradually initiating collagen synthesis (Ananta, 2020).

Fourth, in the maturation phase, the flavonoid content in the nanoemulgel ethanol extract of Dayak onion can suppress the inflammatory phase and accelerate the proliferation phase. To maximize the maturation phase, the wound undergoes a maturation process in which collagen and elastin fibers are continuously stored and reshaped along with changes from fibroblasts to myofibroblasts. The change from fibroblasts to myofibroblasts causes the wound tissue to contract and stretch to minimize the wound surface area until the granulation tissue changes to scar tissue.

Another study conducted by Khairiah and Kusuma in 2021 about anti-Inflammatory activity of Dayak onion ethanol extract cream (*Eleutherine Palmifolia* (L) Merr) on male White rat wounds found that the preparation of Dayak onion ethanol extract cream had strong anti-inflammatory properties. This made the cuts heal faster, and by day 12, they were completely closed. Formula 3, containing Dayak onion ethanol extract at a 15% concentration, demonstrated the most effective anti-inflammatory activity for healing incision wounds. Utilizing nanotechnology in the form of nanoparticles has been shown to enhance pharmacological effects. In the present study, various concentrations of Dayak onion bulb extract (2.5%, 5%, and 7.5%) exhibited biopsy wound healing activity on days 13 and 14, resulting in 100% wound closure. This finding highlights that nanoemulgel formulations with lower concentrations can yield similar efficacy compared to higher concentrations utilized in previous studies.

Statistical analysis using IBM SPSS Statistics 29 (IBM Corp., Chicago, IL.) paired T-test results were obtained for groups III, V, and VI compared to groups I and II, $p < 0.05$, which means that the speed of healing of biopsy wounds in rats with different treatments shows a significant difference. Whereas for group IV, compared to groups I and II, the paired t-test results are $p > 0.05$, which means that the speed of healing of biopsy wounds in rats with different treatments does not show a significant difference.

CONCLUSIONS

According to the Factorial Box Behnken design test method, the best nanoemulsion was made from the first partial derivative with 22.5 mL of Tween 80 and 2 mL of isopropyl myristate. The average globule diameter was 12.13 nm, and 99.12% of the light passed through. The testing to see how well nanoemulsion gel preparations of *Eleutherine palmifolia* extract heal wounds showed that they can be used for aiding in healing and treating biopsy wounds. The effect of biopsy wound healing showed the most effective results were 5% concentrations, with a faster wound healing time of 13 days.

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CONFLICT OF INTEREST

All authors declared no conflict of interest.

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