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RESEARCH ARTICLE

D-Optimal Design-Based Formulation and Evaluation of Tamanu Oil (*Calophyllum inophyllum* L.) Emulgel for Open Wound Healing and Erythema Reduction in Male Wistar Rats

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SUMMARY

Tamanu oil (Calophyllum inophyllum L.) is recognised for its wound-healing potential; however, its oily consistency and formulation instability restrict its topical application. To address these limitations, this study aimed to develop and optimise a stable tamanu oil-based emulgel using a D-optimal mixture design in Design Expert® software. It then evaluated its wound healing activity in an open wound model. The formulation was optimised by varying the concentrations of hydroxypropyl methylcellulose (HPMC) and propylene glycol, with pH and viscosity as response variables. The selected formulation (24.72% HPMC and 75.28% propylene glycol) was assessed in vivo in male Wistar rats. Wound healing progression was monitored over 21 days and analysed using ImageJ. Percentage wound closure and erythema duration were statistically evaluated using one-way ANOVA, followed by LSD post hoc analysis for wound closure (AUC-based) and Duncan's test for erythema. The optimised emulgel showed favourable physicochemical properties and maintained stability over four weeks. In vivo, it achieved 96.66% wound closure by day 15 and complete closure by day 21, with a significantly lower AUC (6.53 ± 1.08; $p < 0.05$) than the control groups. Erythema resolved more rapidly in the tamanu group, supporting its anti-inflammatory potential.

Keywords: *Calophyllum inophyllum, D-optimal design, emulgel formulation, tamanu oil, wound healing.*

Erkek Wistar Sıçanlarında Açık Yara İyileşmesi ve Eritem Azaltılması İçin Tamanu Yağı (Calophyllum Inophyllum L.) Emülgelinin D-Optimal Tasarım Temelli Formülasyonu ve Değerlendirilmesi

ÖZ

Tamanu yağı (Calophyllum inophyllum L.), yara iyileştirici potansiyeli ile tanınmaktadır; ancak yağlı yapısı ve formülasyon kararsızlığı, topikal uygulama alanını kısıtlamaktadır. Bu sınırlamaları aşmak amacıyla, bu çalışma Design Expert® yazılımında D-optimal karışım tasarımı kullanılarak kararlı bir tamanu yağı bazlı emuljel geliştirmeyi ve optimise etmeyi, ardından açık yara modelinde yara iyileştirici etkinliğini değerlendirmeyi amaçlamıştır. Formülasyon optimizasyonu, hidroksipropil metilselüloz (HPMC) ve propilen glikol konsantrasyonlarının değiştirilmesi ile gerçekleştirilmiş; pH ve viskozite yanıt değişkenleri olarak belirlenmiştir. Seçilen formülasyon (%24,72 HPMC ve %75,28 propilen glikol) erkek Wistar sıçanlarında in vivo olarak test edilmiştir. Yara iyileşme süreci 21 gün boyunca takip edilmiş ve ImageJ yazılımı kullanılarak analiz edilmiştir. Yara kapanma yüzdesi ve eritem süresi, tek yönlü ANOVA ile istatistiksel olarak değerlendirilmiş; yara kapanması (AUC temelli) için LSD post hoc analizi, eritem için ise Duncan testi uygulanmıştır. Optimise edilen emuljel, uygun fizikokimyasal özellikler sergilemiş ve dört hafta boyunca kararlılığını korumuştur. In vivo sonuçlara göre, 15. günde %96,66 oranında yara kapanması ve 21. günde tam kapanma elde edilmiş; kontrol gruplarına kıyasla anlamlı derecede düşük bir AUC değeri (6,53 ± 1,08; $p < 0,05$) gözlenmiştir. Eritem, tamanu grubu ile daha hızlı iyileşmiş olup bu bulgu, ürünün antiinflamatuvar potansiyelini desteklemektedir.

Anahtar Kelimeler: *Calophyllum inophyllum, D-optimal tasarım, emuljel formülasyonu, tamanu yağı, yara iyileşmesi.*

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INTRODUCTION

Wound healing is a multifaceted and tightly regulated biological process involving an orchestrated sequence of overlapping phases, such as hemostasis, inflammation, proliferation, re-epithelialisation, angiogenesis, and tissue remodelling. This physiological response is essential for restoring injured skin's structural and functional integrity (Mansour et al., 2025). However, in excisional open wounds, healing can be delayed or impaired, resulting in chronic wounds, infection susceptibility, and poor tissue regeneration (Maqbool et al., 2025). Therefore, clinical management of wounds demands effective therapeutic agents and delivery systems that enhance healing outcomes while ensuring patient compliance.

One of the critical components in modern wound care is the use of topical formulations or wound dressings designed to protect the wound, provide a moist environment, absorb exudates, and deliver bioactive substances directly to the site of injury (Minsart et al., 2022; Su et al., 2023). To modulate the wound microenvironment, ideal wound care products should offer additional pharmacological benefits, such as anti-microbial, anti-inflammatory, and antioxidant activities (Moradifar et al., 2025). Despite the availability of synthetic wound dressings and pharmaceutical products, the demand for plant-based therapies is increasing due to their affordability, multi-target mechanisms, and traditional validation across various cultures (Elkordy et al., 2021; Nasim et al., 2022).

Calophyllum inophyllum L., widely known as tamanu, is a tropical tree native to Southeast Asia and the Pacific Islands. Its seed-derived oil, tamanu oil, has a long history of use in traditional medicine for treating skin ailments, burns, and wounds (Ferdosh, 2024; Krishnappa et al., 2024). Tamanu oil has been traditionally used by coastal and island communities in Indonesia, Polynesia, and Melanesia as part of their cultural healing practices. In this study, we take an ethnopharmacological approach to examine and improve its therapeutic use through innovative formulation methods. Phytochemical analyses have identified calophyllolide, inophyllums, calanolides, fatty acids (such as oleic acid and linoleic acid), and tamanolides as key bioactive constituents responsible for its therapeutic effects (Ferdosh, 2024). These compounds have demonstrated anti-inflammatory, antibacterial, antioxidant, and tissue-repairing activities in various experimental models. Calophyllolide, for instance, was shown to enhance re-epithelialisation, reduce fibrosis, and stimulate wound closure in rats, outperforming standard treatments such as povidone-iodine (Nguyen et al., 2017). Furthermore, Erdogan et al. (2021) reported that topical application of tamanu oil in full-thickness wound models improved collagen density and accelerated wound contraction. Additionally, cold-pressed tamanu oil is rich in unsaturated fatty acids such as oleic and linoleic acids (Rakhmawati et al., 2024a), which are known to promote keratinocyte migration, modulate inflammation, and accelerate dermal tissue regeneration (Tarigan et al., 2024; Wang et al., 2024).

While these findings highlight the therapeutic promise of tamanu oil, its direct application in clinical or experimental settings poses certain limitations. The oil is inherently greasy, sticky, and difficult to spread uniformly, which hinders patient comfort and dosage accuracy. Moreover, the physical instability of tamanu oil in its native form raises concerns regarding its shelf life and consistency during topical use (Krishnappa et al., 2024). Incorporating tamanu oil into an emulgel-based delivery system represents a rational formulation strategy to address these challenges. Emulgels combine the emollient and solubilising properties of emulsions with the non-greasy, bioadhesive, and cooling features of gels, thereby offering a favourable vehicle for lipophilic natural products (Ganju et al., 2024; Kumbhar et al., 2025). Emulgels are particularly advantageous for dermal applications, as they enhance drug permeation through the skin, provide sustained release, and improve user acceptability (Suena et al., 2024).

Despite tamanu oil's well-established wound healing potential and the growing interest in emulgel-based topical systems, studies exploring its incorporation into optimised formulations tailored for excisional open wounds remain limited. Previous research has primarily focused on evaluating the wound-healing activity of pure tamanu oil in incision wound models, whereas the present study investigates its potential in open wound healing. For instance, Rakhmawati et al. (2024a) demonstrated that cold-pressed tamanu oil exhibited superior fatty acid characteristics and significantly enhanced wound closure in vivo compared to hot-pressed tamanu oil and controls. In a separate study, the same authors further confirmed the efficacy of tamanu oil in promoting wound healing in diabetic rat models, highlighting its potential as a natural therapeutic agent (Rakhmawati et al., 2024b). However, these investigations evaluated tamanu oil solely in its unformulated state, overlooking the critical role of delivery systems in maximising therapeutic outcomes. Without an optimised formulation, challenges remain in ensuring sufficient skin permeation, maintaining stability, and enhancing user comfort during application. Moreover, while the anti-inflammatory properties of tamanu oil are widely recognised, its impact on erythema, a common clinical indicator of inflammation during the early stages of wound healing (Johnson et al., 2024), has not been sufficiently investigated in controlled animal studies. This represents an important knowledge gap, particularly considering that erythema, if prolonged or excessive, may signal persistent inflammation and delayed wound resolution (Roșca et al., 2025). Therefore, further investigation into the efficacy of tamanu oil in modulating both wound closure and erythema through a stable and practical emulgel delivery system is essential to realise its potential as a natural topical therapeutic agent fully.

Therefore, this study aimed to optimise a stable and practical tamanu oil-based emulgel formulation using a D-optimal design approach within the Design Expert® software. This approach, grounded in response surface methodology (RSM), enables efficient exploration of formulation variables through minimal experimental runs while evaluating each factor's individual and interactive effects. Furthermore, the optimised formulation was evaluated for its therapeutic performance in wound closure and erythema reduction using an excisional open wound model in male Wistar rats.

MATERIALS AND METHODS

Materials

Cold-pressed *Calophyllum inophyllum* (tamanu) oil was used as the active ingredient. Hydroxypropyl methylcellulose (HPMC; Brataco) served as the gelling agent, while propylene glycol (Brataco) functioned as the humectant. Additional excipients included liquid paraffin (Brataco) as the emollient, Tween 80 and Span 80 (Brataco) as surfactants, phenoxyethanol as a preservative, and menthol as a fragrance-correcting agent. Distilled water was used as the solvent. Commercial product gel was used as the positive control. Other materials included alcohol swabs, surgical scalpels (B-Braun), and BR® rodent feed.

Animals

Twenty-four healthy male Wistar rats (150–200 g, 2–3 months old) were obtained from the Laboratory of Animal Development, LIPI, Yogyakarta. Animals were acclimatised for seven days in cages containing 2–3 cm thick husk bedding, which was replaced every three days. Rats were fed 15 g of standard chow daily. Ethical approval was obtained from the Ethics Committee of Universitas Gadjah Mada (Approval No. 110/UN27.06.11/KEP/EC/2022).

Emulgel formulation and optimization

Based on preliminary tests, Tamanu oil emulgel was formulated using HPMC and propylene glycol in a 1:5 ratio (w/w). Emulsification was achieved using a combination of Tween 80 and Span 80, forming an oil-in-water (o/w) emulsion system. A D-optimal mixture design was employed using Design Expert® software (version 9, Stat-Ease Inc.) to optimise the

concentrations of HPMC and propylene glycol. Thirteen formulations were generated, and responses were evaluated based on pH and viscosity. The optimal formulation was selected based on the desirability index (value = 1) and verified using a one-sample t-test. A formulation was considered valid if the experimental results did not significantly differ from the predicted values ($p > 0.05$).

Evaluation of the physicochemical properties of the optimised emulgel

The optimised formulation was evaluated for organoleptic characteristics, homogeneity, pH, viscosity, spreadability, and adhesion. Homogeneity was assessed microscopically by spreading the gel between two glass slides and observing the distribution of particles. The pH was measured using a digital pH meter, with the acceptable range for topical application being 4.5 to 6.5 to ensure compatibility with the skin. Viscosity was determined using a Rion viscometer equipped with spindle number 2, where an ideal range between 50 and 1000 dPas was considered acceptable for semi-solid preparations (Tahar et al., 2023). Spreadability was evaluated by placing 0.5 g of the emulgel between two glass plates under a 150 g load for one minute, and the resulting spread diameter was measured; values between 5 and 7 cm were deemed acceptable. Adhesion was assessed using a glass-slide assembly, where the time required for the upper slide to detach under an 80 g load, following an initial loading of 1 kg for five minutes, was recorded as the adhesion time (Rahmani & Zulkarnain, 2023).

Evaluation of open wound healing activity

The wound healing activity of the optimised tamanu oil emulgel was evaluated using an open excision wound model in male Wistar rats. The number of animals per group was determined using Federer's formula to ensure statistical validity, as follows (Tahar et al., 2023):

$$(t - 1)(n - 1) \geq 15$$

With four treatment groups (t = 4), the minimum number of animals per group was calculated to be at least six ($n \geq 6$), resulting in a total of 24 animals randomly assigned to each group.

Before wound induction, the rats were anesthetised with ketamine, and the dorsal fur was shaved and disinfected using 70% ethanol. A 1 cm × 1 cm excision wound with a depth of 2 mm was created on the upper dorsal region using a sterile scalpel, limited to the dermal layer without damaging the underlying muscle, following the method of Rakhmawati et al. (2024a). The treatment formulations (0.5 g) were applied topically once daily for 21 consecutive days.

The treatment groups included a normal control (untreated), a negative control (gel base without tamanu oil), a positive control (commercial product®), and the test group, which received 20% (w/w) tamanu oil emulgel for its wound healing potential (Krishnappa et al., 2024).

Daily visual observation assessed changes in wound colouration (from red to white), scab formation, and re-epithelialisation. Wounds were photographed on days 0, 3, 6, 9, 12, 15, 18, and 21 using a fixed camera distance and scale for quantitative analysis of wound area using ImageJ software.

Data analysis

Digital photographs of wounds were analysed using ImageJ software. The wound healing progress was calculated as percent wound closure using the following formula (Rakhmawati et al., 2024a):

$$\%P = \frac{(do - dx)}{do} x100$$

Where:

do= initial wound area (day 0)

dx = wound area on day x

The total area under the curve (AUC) of wound area over time was calculated to reflect overall wound healing dynamics across the study period, using the following rule:

$$\underset{tn-1}{\overset{tn}{AUC}} = \frac{L_{tn-}1 + L_{tn}}{2} \times (t_n - t_{n-1})$$

Where:

L_{tn} and L_{tn-1}= mean wound area at time t_n and $t_{n-1,}$ respectively.

All data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS® version 21.0. Wound closure percentage and AUC values were analysed using one-way ANOVA, followed by Least Significant Difference (LSD) post hoc tests if p-values were below 0.05. A significant difference ($p < 0.05$) compared to the control groups indicated wound healing efficacy.

Additionally, time to erythema resolution was recorded through daily macroscopic observation for 21 days. These data were also analysed using one-way ANOVA, followed by Duncan's multiple range test if ANOVA indicated significant differences ($p < 0.05$).

RESULTS AND DISCUSSION

Optimisation of tamanu oil (*Calophyllum inophyllum* L.) emulgel formulation

The optimisation of tamanu oil emulgel formulation aimed to develop a physically stable and pharmaceutically acceptable topical dosage form. This study employed a combination of Tween 80 (1.08%) and Span 80 (0.42%) as emulsifying agents, resulting in a hydrophilic–lipophilic balance (HLB) value of 12.004, which supports the formation of oil-in-water (O/W) emulsions. O/W emulsions are preferred for topical applications due to their better patient acceptability, non-greasy feel, and easier washability (Ali et al., 2022). The overall formulation for five trial batches (F1–F5) is presented in Table 1.

Table 1. Formulation of tamanu oil emulgel formulations (75 g per tube) for trial batches F1–F5

Ingredients	Concentration of Ingredients in Gel Formula (g)				
	F1	F2	F3	F4	F5
Tamanu oil	15	15	15	15	15
HPMC	0.75	1.5	2.25	3	3.75
Propylene glycol	3.75	3	2.25	1.5	0.75
Parafin liquidum	3.75	3.75	3.75	3.75	3.75
Tween 80	0.81	0.81	0.81	0.81	0.81
Span 80	0.315	0.315	0.315	0.315	0.315
Phenoxyetanol	0.375	0.375	0.375	0.375	0.375
Menthol	0.75	0.75	0.75	0.75	0.75
Aquadest	ad 75	ad 75	ad 75	ad 75	ad 75

A key component of the formulation optimisation was the selection of appropriate concentrations of HPMC (hydroxypropyl methylcellulose) as a gelling agent and propylene glycol as a cosolvent and humectant. These two components were selected as independent variables in a mixture design optimisation using the D-optimal method in Design Expert 9 software. The design space was defined with HPMC ranging from 16.67% to 33.33%, and propylene glycol from 66.67% to 83.33%, keeping the total proportion of both components at 100%.

The experimental design generated 13 run formulations with varying HPMC and propylene glycol ratios, while maintaining the total concentration of both components at 100%. Several formulations shared identical compositions, such as run 2 with runs 8, 11, and 13; run 3 with runs 9 and 12; and run 4 with runs 5, 6, and 10. This repetition reflects the software's strategy in placing replicates at critical design points, particularly at the centre or vertices of the design space, to evaluate model robustness, minimise experimental error, and improve the reliability of the response surface model. Each formulation was evaluated for pH and viscosity, essential parameters for topical emulgels. The detailed composition of each run and the corresponding response values for pH and

viscosity are presented in Table 2. A pH within the range of 5.5 to 6.5 is considered compatible with the skin and avoids irritation (Khan et al., 2024), while viscosity affects the preparation's spreadability, retention, and overall patient compliance (Humaira et al., 2025).

Table 2. Experimental design matrix and observed viscosity values for tamanu oil emulgel formulations based on D-optimal mixture design involving HPMC and propylene glycol

Run	A: HPMC (%)	B: Propylene glycol (%)	Parameter	
			pH	Viscosity (dPas)
1.	20.835	79.165	6.42	490
2.	33.330	66.670	6.36	600
3.	25.000	75.000	6.40	495
4.	16.670	83.330	6.44	253
5.	16.670	83.330	6.45	420
6	16.670	83.330	6.44	490
7.	29.165	70.835	6.37	595
8.	33.330	66.670	6.35	650
9.	25.000	75.000	6.41	450
10.	16.670	83.330	6.44	460
11.	33.330	66.670	6.36	645
12.	25.000	75.000	6.40	410
13.	33.330	66.670	6.34	660

The response analysis showed that both pH and viscosity followed linear models with statistically significant effects from the variation in HPMC and propylene glycol concentrations ($p < 0.05$) (Figures 1 and 2.). The lack-of-fit test results were non-significant ($p = 0.56$ for pH, $p = 0.47$ for viscosity), indicating that the linear models adequately predicted the observed responses.

The optimal formulation was determined using a desirability function approach, yielding a value of 0.787. Although not reaching the theoretical maximum of 1.0, this score represents a highly acceptable result within pharmaceutical formulation standards. In D-optimal mixture designs, desirability values above 0.7 are typically considered robust and indicate a satisfactory balance between multiple responses (Araghi et al., 2023). The value of 0.787 thus reflects the best achievable compromise between pH and viscosity within the studied design space.

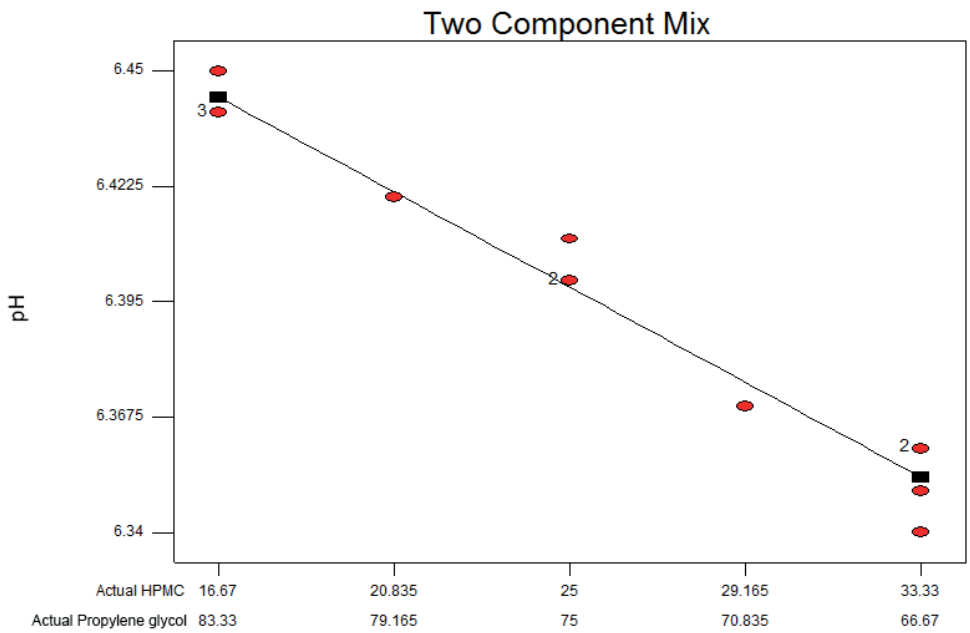

Figure 1. Response surface plot showing the effect of HPMC and propylene glycol concentrations on the pH of tamanu oil emulgel formulations

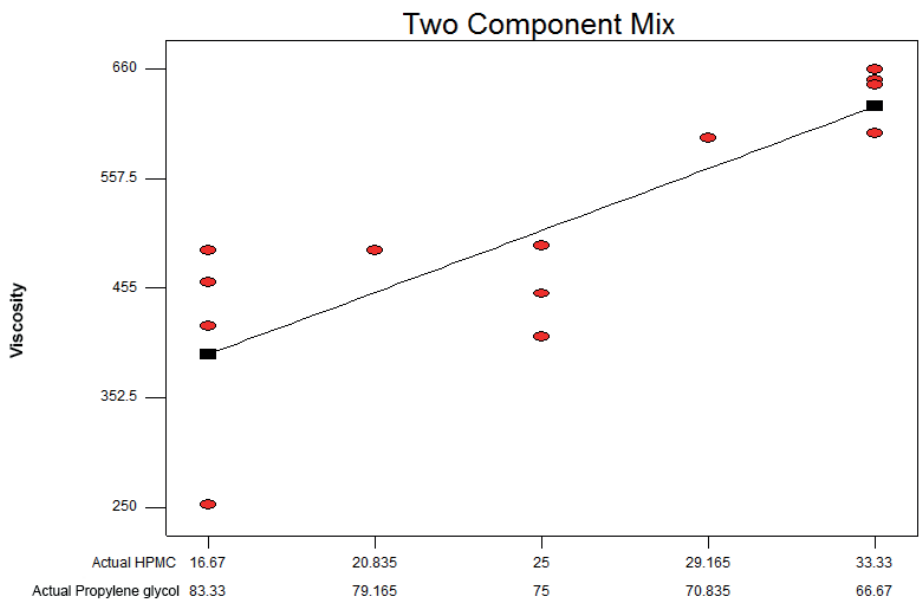

Figure 2. Response surface plot illustrating the influence of HPMC and propylene glycol concentrations on the viscosity of tamanu oil emulgel formulations

This optimal formula comprised 24.72% HPMC and 75.28% propylene glycol, with predicted values of pH 6.39 and viscosity 505.18 dPas (Table 3.). The formulation was subsequently verified in triplicate, showing observed values (mean ± SD) of pH 6.39 ± 0.031 and viscosity 498.33 ± 7.64 dPas, with no statistically significant difference from predicted values ($p>0.05$, Table 4.).

Table 3. Predicted optimal formulation of tamanu oil emulgel and corresponding response values (pH and viscosity) generated by Design Expert® software

HPMC (%)	Propylene glycol (%)	pH	Viscosity	Desirability
24.72	75.28	6.39	505.18 dPas	0.787

Table 4. Predicted optimal formulation of tamanu oil emulgel and corresponding response values (pH and viscosity) generated by Design Expert® software

Parameter	Prediction	Observation				*p-value*
		R1	R2	R3	Mean ± SD	
pH	6.39	6.38	6.42	6.36	6.39 ± 0.031	0.528
Viscosity (dPas)	505.18	505	490	500	498.33 ± 7.637	0.264

R1= Replication 1; R2= Replication 2; R3= Replication 3

This combination was deemed optimal because it produced a gel with acceptable viscosity and pH, ensuring ease of application, adequate retention on the skin, and safety for prolonged topical use. A balance between HPMC and propylene glycol is critical, as excessive HPMC increases viscosity excessively, impairing spreadability (Narulita et al., 2024), while excess propylene glycol can lower viscosity and compromise emulgel structure (Otake et al., 2025).

In addition to their functional roles, HPMC and propylene glycol also contribute to formulation stability. HPMC serves as a viscosity enhancer and helps stabilise emulsions by forming a three-dimensional gel matrix that entraps oil droplets, preventing phase separation and improving shelf-life (Ferdaus et al., 2025). Propylene glycol, on the other hand, improves sensory feel and acts as a penetration enhancer, which is beneficial for topical delivery systems (Mistry & Notman, 2024).

These findings are supported by Andina et al. (2021), who optimised a green tea gel formulation using the D-optimal method and found that increasing HPMC concentration significantly raised viscosity, while propylene glycol functioned as a viscosity-modifying and spreadability-enhancing agent. Their study demonstrated that a careful balance between these two components is essential to achieve desirable gel characteristics, aligning with the current formulation approach.

Physicochemical performance of the optimised emulgel

The optimised tamanu oil emulgel exhibited favourable physical and physicochemical characteristics over a four-week storage period (see Table 5). Organoleptically, the formulation retained a stable greenish colour, semi-solid consistency, and characteristic tamanu scent throughout the observation period. The absence of coarse particles under microscopic evaluation further confirmed the homogeneity and colloidal stability of the formulation, suggesting effective emulsification and polymer dispersion (Bruno et al., 2022; Silva et al., 2016).

Table 5. Physicochemical characteristics of the optimised tamanu oil emulgel over 4 weeks of storage (mean±SD)

Week	pH	Viscosity (cPs)	Spreadability (cm)	Adhesiveness (s)
1	6.42±0.33	498.33 ± 7.64	5.87 ± 0.15	3.86 ± 0.41
2	6.32±0.35	473.33 ± 15.28	6.33 ± 0.43	3.47 ± 0.43
3	6.24±0.43	453.33 ± 15.28	6.67 ± 0.55	3.11 ± 0.28
4	6.18±0.41	431.67 ± 10.41	7.28 ± 0.43	2.85 ± 0.46

The pH values remained within the physiologically acceptable range (6.18–6.42), showing no statistically significant difference over time ($p > 0.05$). Maintaining a pH within this range is crucial for dermal compatibility, as formulations between 5.5 and 6.5 preserve the skin barrier's integrity and minimise irritation risk (Khan et al., 2024). Previous studies have shown that pH values exceeding the skin's natural pH may alter enzymatic activity in the stratum corneum, potentially disrupting barrier function and microbiome balance (Kuo et al., 2020; Rajkumar et al., 2023).

A key physicochemical change was observed in the viscosity, which showed a statistically significant decline from 498.33 ± 7.64 cPs in week 1 to 431.67 ± 10.41 cPs in week 4 ($p = 0.001$). The observed decrease in viscosity over the four-week storage period may be attributed to the gradual weakening of the HPMC gel network. Ding et al. (2014) reported reduced molecular interaction in HPMC-based systems can lower viscosity. Noval et al. (2020) found that storage led to viscosity decline in HPMC gels due to changes in hydrogen bonding and flow behaviour. These findings suggest that storage conditions likely contributed to the reduced structural integrity of the emulgel matrix. Despite the reduction, the viscosity remained within the optimal range for semi-solid topical systems, which ensures sufficient retention at the application site and controlled drug release. These viscoelastic properties are crucial for maintaining formulation integrity while allowing user-friendly application (Botan et al., 2024).

The spreadability significantly increased from 5.87 ± 0.15 cm in week 1 to 7.28 ± 0.43 cm in week 4 ($p = 0.018$), correlating inversely with the observed decrease in viscosity. As the internal gel structure relaxes, less force is required to spread the formulation, resulting in improved spreadability. This property is essential to ensure uniform distribution of the active ingredient over the skin surface and improve patient compliance. An increase in spreadability also enhances the surface area for drug absorption, thereby potentially improving therapeutic efficacy (Ding et al., 2014).

The adhesion time decreased slightly over the storage period, from 3.86 ± 0.41 to 2.85 ± 0.46 seconds, although this change was not statistically significant ($p > 0.05$). This trend follows the viscoelastic behaviour of the gel, where reduced viscosity and lower gel strength diminish the formulation's ability to resist detachment from the skin (Rahayu et al., 2024). Nevertheless, the final adhesion value remained acceptable for topical application (greater than one second), indicating that the gel could maintain contact long enough to facilitate drug permeation (Ridwanto et al., 2023).

These findings indicate that the optimised emulgel formulation achieved a suitable balance of physicochemical characteristics essential for dermal delivery. The stable pH and viscosity values suggest good formulation robustness, while the improved spreadability and maintained adhesion highlight its potential for enhanced patient compliance and therapeutic performance.

Wound healing activity in the excisional open wound model

The wound healing activity of the optimised tamanu oil emulgel was evaluated over 21 days using an excisional open wound model in male Wistar rats. Open wounds are injuries involving a break in the skin barrier, exposing underlying tissue to potential infection and delaying the healing process if not properly managed (Sharma et al., 2021). Morphologically (Figure 3A), the wounds in all treatment groups gradually healed from day 1 to day 21. On day 1, all rats exhibited fresh, open wounds with visible erythema and swelling. On day 10, scab formation was evident in all groups. However, the tamanu oil emulgel group displayed smaller scabs with well-defined edges, suggesting active re-epithelialisation (Cioce et al., 2024). By day 15, the emulgel-treated wounds had lost most of their scabs, revealing newly formed epithelial tissue with minimal signs of inflammation. In contrast, wounds in the untreated and negative control groups retained larger scabs and incomplete closure. Com-

plete epithelialisation was observed in the emulgel group by day 21, where wound areas were fully closed and indistinguishable from surrounding skin (Liu et al., 2025).

The percentage of wound closure was calculated based on the reduction in wound size over time. Figure 3B shows the average wound closure percentage across all groups over the 21 days. Tamanu oil emulgel group exhibited the fastest and most complete wound closure, achieving 96.66% closure by day 15 and 100% closure by day 21. In contrast, none of the control groups reached complete closure by day 21, with the positive control showing intermediate efficacy, outperforming the negative and normal controls, but not surpassing the tamanu oil emulgel.

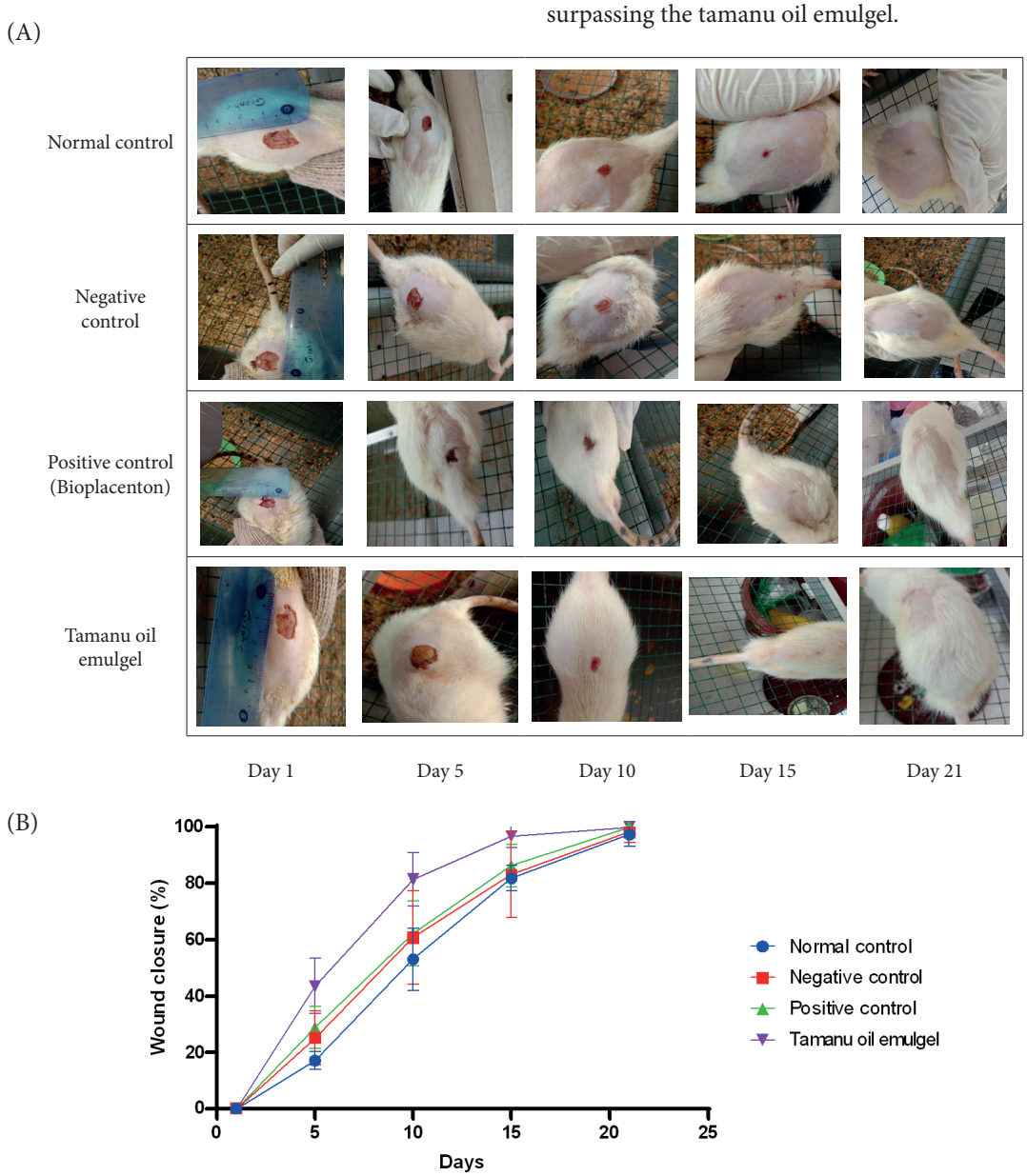

Figure 3. *Macroscopic evaluation and quantification of wound closure over 21 days.* (**A**) Representative macroscopic images of wound healing progression in rats (n = 6 per group) from each treatment group on days 1, 5, 10, 15, and 21 following wound excision. (**B**) Percentage of wound closure (mean ± SD, n = 6) at each observation point (days 1, 5, 10, 15, and 21) across all groups.

The wound healing progression in this study aligns with the typical phases of cutaneous wound repair, which consist of inflammation (days 0–4), proliferation (days 5–21), and remodelling (beyond day 21) (Gupta et al., 2023; Nasiry et al., 2022). On day 1, all groups showed minimal closure, reflecting the early inflammatory response of edema and erythema. By day 5, the tamanu oil emulgel group exhibited a notably higher wound closure (43.56%) than controls, suggesting an accelerated transition into the proliferative phase, likely driven by anti-inflammatory and regenerative phytochemicals in tamanu oil. Continued improvements were observed on days 10 and 15, where the emulgel group consistently outperformed other treatments, reaching 81.29% and 96.66% wound closure, respectively. These findings suggest that the optimised emulgel formulation enhances active compound delivery and supports sustained fibroblast activity, collagen synthesis, and re-epithelialisation throughout the proliferative phase. By day 21, near-complete healing (100%) was observed in the tamanu group, indicating a potential role of the formulation in promoting faster tissue remodelling (Cioce et al., 2024; Stojanovic et al., 2024). The prolonged inflammatory signs and delayed scab shedding in other groups suggest a slower resolution of inflammation and suboptimal transition to the proliferative phase, which may explain their lower wound closure percentages (Binsuwaidan et al., 2024).

To comprehensively assess the wound healing performance of the tested formulations, the area under the curve (AUC) of wound size reduction was calculated over the 21-day treatment period. AUC serves as an integrated indicator of the healing trajectory, capturing both the rate and extent of wound closure over time, rather than relying solely on endpoint measurements (Lammert et al., 2024). As shown in Table 6, the tamanu oil emulgel group exhibited the lowest total AUC, indicating a consistently rapid wound contraction and superior healing kinetics. Statistical one-way ANOVA analysis demonstrated significant differences among the treatment groups ($p = 0.009$). Post hoc LSD tests confirmed that the tamanu oil emulgel significantly outperformed both the normal and the negative control groups ($p < 0.05$), although no statistically significant difference was observed when compared to the Bioplacenton® group.

This finding demonstrates that the tamanu oil emulgel in this study has a notably lower AUC value than the cold-pressed tamanu oil reported by Rakhmawati et al. (2024a), which was 11.67 ± 0.78. The marked decrease in AUC reflects a significantly enhanced wound healing rate, underscoring the superior efficacy of the emulgel formulation over the unformulated oil. This improvement can be attributed to the emulgel's ability to enhance skin permeation and retention of active compounds and its improved physicochemical stability and patient acceptability (Suena et al., 2024). Collectively, these factors contribute to more efficient and sustained delivery of tamanu oil's bioactive components, thereby accelerating the wound closure process and optimising therapeutic outcomes.

Table 6. The area under the curve (AUC) of the wound area is over 21 days for each treatment group. Data are presented as individual values per animal and as mean ± standard deviation (SD).

Treatment Group	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SD
Normal Control	9.52	8.30	11.41	10.31	10.54	10.36	10.07±1.06 [a]
Positive Control	7.86	9.59	10.13	7.33	6.13	9.40	8.40 ± 1.55
Negative Control	12.12	10.89	8.27	8.21	6.58	6.62	8.78 ± 2.27 [a]
Tamanu Oil Emulgel	7.24	6.94	7.48	4.46	6.47	6.65	6.53 ± 1.08[a]

Superscript letter [a] indicates that the tamanu oil emulgel group showed a statistically significant difference ($p < 0.05$) compared to the normal control and negative control groups based on LSD post hoc analysis.

In addition to wound size, erythema resolution was monitored as a marker of inflammation attenuation. Erithema represents the visible manifestation of localised vascular response and leukocyte recruitment during the inflammatory phase of wound healing, typically peaking within the first four days post-injury (Pan et al., 2022; Wong et al., 2023). The average duration for erythema disappearance in the tamanu oil emulgel group was 9.5 days (Table 7.). One-way ANOVA followed by Duncan's post hoc test confirmed statistically significant differences ($p < 0.05$) between the tamanu oil group and the normal and negative control groups. However, there was no significant difference between the tamanu oil group and the positive group, indicating comparable anti-inflammatory performance.

Table 7. Duration (in days) required for erythema disappearance at wound sites in each treatment group. Data are presented as individual values per animal and as mean ± standard deviation (SD).

Treatment Group	Duration of Wound Erythema Resolution (Days)						
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean ± SD
Normal control	15	14	18	17	14	16	15.67 ± 1.63 [a]
Positive control	13	19	18	10	10	16	14.33 ± 3.87
Negative control	19	16	14	10	12	12	13.83 ± 3.06 [a]
Tamanu oil emulgel	7	11	10	7	12	10	9.50 ± 1.87[a]

Superscript letter [a] indicates that the tamanu oil emulgel group showed a statistically significant difference ($p < 0.05$) compared to the normal control and negative control groups based on Duncan post hoc analysis

Several recent studies have explored the wound healing potential of tamanu oil, highlighting its promising pharmacological activities in skin regeneration, including anti-inflammatory, anti-microbial, and anti-erythema effects. Ansel et al. (2016) demonstrated that tamanu oil extract promoted wound repair in human keratinocytes and dermal fibroblasts by upregulating genes associated with cell adhesion, proliferation, and O-glycan biosynthesis. Building on this, Nguyen et al. (2017) isolated calophyllolide, one of tamanu oil's major bioactive components, and reported its ability to reduce fibrosis, shift macrophage polarisation toward the M2 phenotype, and enhance complete re-epithelialisation within 14 days in excisional wound models. In a subsequent in vivo study, Erdogan et al. (2021) observed a significant improvement in wound contraction in rats treated with tamanu oil, alongside increased macrophage infiltration and more mature granulation tissue formation by day 7. Rakhmawati et al. (2024a) further confirmed the efficacy of cold-pressed tamanu oil, which exhibited the fastest wound healing rate among all treatment groups, as indicated by the lowest area under the curve (AUC) value. This result was supported by its favourable physicochemical properties, namely lower acid and peroxide values, as well as its higher content of linoleic and oleic acids. These fatty acids have been implicated in key mechanisms of tissue repair. Oleic acid has been shown to modulate inflammatory responses by increasing IL-10 expression and reducing COX-2 levels, leading to enhanced collagen deposition and balanced cytokine activity (Cardoso et al., 2011). Similarly, linoleic acid accelerated wound closure in porcine burn models, with complete healing observed in 37 days compared to 45 days in untreated controls (Zhao et al., 2022). Both fatty acids are believed to support tissue remodelling through the regulation of matrix metalloproteinase (MMP) activity, further underscoring their contribution to the wound healing process.

Despite this growing body of evidence, the application of *Calophyllum inophyllum* oil in statistically optimised delivery systems for open wound healing remains limited. However, the application of *Calophyllum inophyllum* oil in optimised delivery systems for open wound healing remains scarcely investigated. This study presents novel findings on formulating a ta-

manu oil emulgel using a D-optimal design approach, emphasising its potential as a scientifically designed, plant-based topical therapy. To our knowledge, this is the first investigation to evaluate such a formulation in an excisional wound model, supporting its promise as a natural and effective candidate for open wound management.

An emulgel system likely enhanced the formulation's effectiveness by improving skin adhesion, drug retention, and user acceptability. Its ability to deliver lipophilic compounds while maintaining a moist wound environment may have supported tissue regeneration more efficiently than conventional preparations (Afzal et al., 2023; Milutinov et al., 2023).

Despite the promising outcomes observed, such as accelerated wound closure and shorter erythema duration, this study is limited to macroscopic evaluations without histological or molecular confirmation of healing mechanisms. Further studies are needed to validate the underlying biological pathways and assess long-term safety.

CONCLUSION

This study successfully developed and optimised a tamanu oil-based emulgel using a D-optimal design approach. The selected formulation, 24.72% HPMC and 75.28% propylene glycol, exhibited desirable physicochemical properties, including suitable pH, viscosity, spreadability, and physical stability for up to four weeks. In vivo testing in male Wistar rats with excisional wounds showed that the optimised emulgel achieved 96.66% wound closure by day 15, indicating accelerated healing. Complete closure was observed by day 21, with a significantly lower wound AUC than the control groups. Erythema resolved faster in the treated group, suggesting effective inflammation control. These findings demonstrate the synergistic therapeutic effect of tamanu oil's bioactives and the enhanced dermal delivery afforded by the emulgel system.

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AUTHOR CONTRIBUTION STATEMENT

RR: Concept and design, drafting the manuscript, supervision, final approval; ANA: Concept and design, data analysis, drafting the manuscript, final approval; RA: data acquisition, statistical analysis, drafting manuscript; KH: data acquisition, drafting manuscript; FHP: data analysis, critical revision of the manuscript, final approval; NH: critical revision of the manuscript, final approval.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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