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# Impact of Emulsifier Balance on Physicochemical Stability and Antiinflammatory Efficacy of *Calotropis gigantea* Extract Emulgel

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**ABSTRACT:** The long-term use of topical non-steroidal anti-inflammatory drugs (NSAIDs) is associated with local irritation and disruption of the skin barrier, highlighting the need for safer plant-based alternatives with adequate formulation stability. *Calotropis gigantea* leaves contain flavonoids with reported anti-inflammatory activity; however, their topical performance is limited by poor solubility and instability. This study aimed to evaluate the effect of emulsifier balance on the physical stability and anti-inflammatory efficacy of a *Calotropis gigantea* leaf extract emulgel. Three emulgel formulations were prepared using different ratios of nonionic surfactants Tween 80 and Span 80 (2:8, 5:5, and 8:2) based on hydrophilic–lipophilic balance (HLB) principles. The formulations were evaluated for physicochemical characteristics, accelerated physical stability using a cycling test, and in vivo anti-inflammatory activity using a carrageenan-induced paw edema model in rats. The formulation containing a balanced emulsifier ratio (5:5) exhibited the smallest deviations in viscosity, spreadability, and pH after cycling tests and demonstrated the highest edema inhibition (68.98 ± 1.64%). These results indicate that emulsifier balance plays a critical role in maintaining emulgel stability and directly influences its biological performance. Although chemical stability and dermal safety were not evaluated, this study provides evidence that rational emulsifier selection is essential for improving the effectiveness of herbal topical formulations.

**Keywords**: *Calotropis gigantea*; emulgel; emulsifier balance; physical stabilty; topical anti-inflammatory

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## INTRODUCTION

Inflammation is a protective biological response to tissue injury, infection, ischemia, and chemical irritation; however, persistent inflammation underlies many chronic diseases that require long-term therapy (Gupta et al., 2015). Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage inflammation and pain, but prolonged use, either orally or topically, is associated with adverse effects including gastrointestinal toxicity, cardiovascular risk, and local skin reactions such as dermatitis and irritation (Semis et al., 2021; Touma et al., 2007; Langworthy et al., 2024). Although topical administration reduces systemic exposure, repeated application may still compromise skin barrier integrity and reduce patient adherence. Therefore, there remains a clear clinical need for safer topical anti-inflammatory alternatives that maintain efficacy while minimizing local adverse effects.

Medicinal plants represent a promising source of alternative anti-inflammatory agents. *Calotropis gigantea* leaves have been reported to contain flavonoids and phenolic compounds with anti-inflammatory, antioxidant, and wound-healing activities (Alafnan et al., 2021). This plant has been traditionally used for the treatment of pain, inflammation, and respiratory disorders (Kumar et al., 2013). However, despite its pharmacological potential, the translation of *Calotropis gigantea* into effective topical dosage forms is limited by poor aqueous solubility and instability of bioactive constituents when incorporated into conventional gels or creams. Formulation strategies that enhance solubility, stability, and skin retention are therefore required to achieve consistent therapeutic outcomes.

Emulgels, which combine the advantages of emulsions and gels, offer an effective platform for topical delivery of hydrophobic herbal extracts by improving solubilization, spreadability, and residence time on the skin (Puspitasari et al., 2023; Prasanth et al., 2025). The performance of emulgels is strongly influenced by the emulsifier system that stabilizes the oil–water interface. Nonionic surfactants such as Tween 80 and Span 80 are frequently used to achieve a balanced hydrophilic–lipophilic balance (HLB) and improve physical stability (Lv et al., 2014). Previous studies have shown that combinations of Tween 80 and Span 80 can produce physically acceptable herbal emulgels with suitable viscosity, pH, and spreadability (Daud & Suryanti, 2017; Rusli et al., 2021). However, most of these studies focus primarily on physicochemical optimization and do not systematically evaluate whether variations in emulsifier ratio translate into differences in biological performance.

As a result, the relationship between emulsifier balance, physical stability under thermal stress, and in vivo anti-inflammatory efficacy remains insufficiently understood. This gap is critical because a formulation that is physically stable does not necessarily ensure consistent therapeutic performance. Therefore, the present study aimed to investigate the effect of emulsifier balance on both the physical stability and anti-inflammatory efficacy of a *Calotropis gigantea* leaf extract emulgel. By integrating accelerated stability testing with in vivo evaluation, this study seeks to provide evidence that rational emulsifier selection is essential not only for maintaining formulation quality but also for ensuring reliable biological performance in topical herbal preparations.

## METHODS

### Plant Material

Fresh leaves were gathered in Bondowoso District, Indonesia, in December 2025. Voucher specimens of *Calotropis gigantea* leaves were authenticated and deposited for future reference under identification number TL.02.04/D.IX.6/31705.1384/2024. The

powdered leaves were extracted by maceration using 70% ethanol as the extraction solvent. The selection of 70% ethanol was based on its intermediate polarity, which allows efficient extraction of flavonoids and phenolic compounds that are reported as the main anti-inflammatory constituents of *C. gigantea* leaves, while also minimizing the co-extraction of excessive nonpolar impurities. Hydroethanolic solvents in this concentration range are widely used for medicinal plant extraction because they provide a balanced yield of polar and moderately polar compounds and are compatible with topical formulation development (Semis et al., 2021). The maceration process was carried out for 3 days with periodic stirring, followed by filtration and solvent removal under reduced pressure using a rotary evaporator at 50°C to obtain a viscous extract. The extraction yield was calculated based on the ratio of dried extract weight to initial dried plant material and expressed as percentage (% w/w) (Ahmad, 2020). The extraction yield obtained was 7.14%.

**Emulgel Preparation**

*Calotropis gigantea* leaves extract was produced using a maceration method and a 70% ethanol solvent. 600 grams of *Calotropis gigantea* leaves powder were soaked in 3L of ethanol in a glass container and tightly sealed for 3 days, stirring occasionally. After three days, *Calotropis gigantea* leaves extract solution was filtered to produce a filtrate and collected. The filtrate obtained was concentrated using a rotary evaporator. The macerate was evaporated with a rotary evaporator at 40°C to get a concentrated extract.

Emulgels are formulated with varied ratios of span 80 and tween 80, as shown in table 1. The selection of Tween 80 and Span 80 ratios (2:8, 5:5, and 8:2) was theoretically based on the hydrophilic–lipophilic balance (HLB) concept. Tween 80, a nonionic surfactant with a high HLB value (HLB=15.0), predominantly stabilizes oil-in-water (o/w) systems by enhancing aqueous phase affinity, whereas Span 80 possesses a low HLB value (HLB=4.3) and favors lipophilic interactions within the oil phase (Lv et al., 2014).

**Table 1.** Formula of emulgel loaded *Calotropis gigantea* leaves extract

| **Material** | **Function** | **Concentration (%)** | | |
|---|---|---|---|---|
| | | **F1** | **F2** | **F3** |
| *Calotropis gigantea* leaves extract | Bioactive ingredients | 5 | 5 | 5 |
| Paraffin Liq | Oil phase | 10 | 10 | 10 |
| Span 80 | Surfactant | 2 | 5 | 8 |
| Tween 80 | Surfactant | 8 | 5 | 2 |
| Propylene Glycol | Humectant | 10 | 10 | 10 |
| Methyl Paraben | Preservative | 0.25 | 0.25 | 0.25 |
| HPMC | Gelling agent | 5 | 5 | 5 |
| Buffer pH 6 ± 0.05 | Water phase | Add 100 | Add 100 | Add 100 |

However, in this study, the ratio variation was designed not merely for incremental optimization but to create emulgel systems with distinct interfacial properties that are expected to influence biological performance. The oil phase was heated to 50°C after being combined with extract, liq paraffin, and span 80. HPMC was combined with pH 6 buffer to create a gel mass prior to combining the water phase. Additionally, a solution of methyl paraben in propylene glycol was added to the gel mass. A temperature equal to that of the oil phase was then applied to the established water phase. After that, the two phases were combined for ten minutes with a mixer. All physicochemical evaluations were performed

in triplicate (n = 3), and data were expressed as mean ± standard deviation to ensure methodological robustness.

**Viscosity Evaluation**

Viscosity was determined using a Brookfield viscometer equipped with a suitable spindle at a constant rotational speed. Measurements were conducted in triplicate, and results were expressed as mean ± SD. For topical emulgels, a viscosity range of 2,000–50,000 cP was defined as acceptable to ensure adequate spreadability, retention on the skin, and patient acceptability (Lv et al., 2014; Puspitasari et al., 2023). Changes in viscosity that remained within this range after stability testing were considered pharmaceutically meaningful, whereas values outside this range were interpreted as indicating loss of formulation integrity. The test sample is placed in a 100 ml beaker glass container, and then the proper spindle is inserted up to the limit mark, turned on, and allowed to revolve. A set number on the scale indicator indicates that the measurement is complete (Rusli et al., 2021).

**pH Evaluation**

The pH test was performed using a pH meter that had been calibrated with standard buffer solutions at pH 4 and pH 7. After calibration, the electrode was placed in a beaker glass containing the emulgel mixture (Puspitasari et al., 2023). The pH of each emulgel formulation was measured using a calibrated digital pH meter at room temperature. Measurements were performed in triplicate, and the mean value was recorded. The acceptable pH range for topical semisolid formulations was defined as 4.5–6.5 to ensure skin compatibility and minimize the risk of irritation during repeated application, as commonly recommended for topical preparations (Daud & Suryanti, 2017; Rusli et al., 2021). Changes in pH within this range were considered pharmaceutically acceptable, while values outside this range were regarded as potentially affecting skin tolerance.

**Stickiness Evaluation**

Stickiness was evaluated by measuring the time required for two glass plates to separate under a defined load. Measurements were performed in triplicate, and results were expressed as mean values. An acceptable stickiness time of ≥1 second was defined to ensure sufficient residence time on the skin without causing discomfort or difficulty during application, in accordance with previously reported criteria for topical semisolid formulations (Rusli et al., 2021). Values below this threshold were considered insufficient for effective topical retention. Emulgel was weighed up to 1 gram and placed between two glass items, then covered and subjected to a load weighing 200 grams for 5 minutes, following which the load was removed and the time required to separate the two glass objects was measured.

**Spreadability Evaluation**

Spreadability was evaluated using the parallel-plate method with a fixed weight applied for a defined period. The diameter of spread was measured in centimeters and recorded as the mean of three determinations. A spreadability range of 5–7 cm was defined as optimal for topical semisolid preparations to ensure ease of application and uniform dosing on the skin surface (Daud & Suryanti, 2017). Variations within this range were considered acceptable, while values outside this range were considered to reduce patient usability. Emulgel was weighed up to 500 grams, then placed on a glass plate, left for a moment, and then loaded with 200 grams; after one minute, the diameter was measured (Ikhsanudin & Azizah, 2019).

**Stability Evaluation**

The physical stability of the emulgel formulations was evaluated using a cycling test as a preliminary stability assessment. Each formulation was subjected to six cycles of

alternating storage at low temperature (4 ± 2 °C) and high temperature (40 ± 2 °C), with each temperature maintained for 24 hours. After completion of the cycling test, the formulations were evaluated for changes in pH, viscosity, spreadability, and stickiness to detect signs of phase separation, structural breakdown, or loss of consistency, as commonly applied for early-stage topical formulation screening (Daud & Suryanti, 2017; Rusli et al., 2021).

In the present study, stability evaluation was limited to physical parameters because the primary objective was to investigate the influence of emulsifier balance on formulation integrity and performance-related attributes. Physical instability, such as viscosity loss or phase separation, is considered an early indicator of potential long-term instability, as it reflects disruption of the emulsion microstructure and interfacial film formed by the emulsifier system (Lv et al., 2014). Therefore, assessment of physical stability under thermal stress provides relevant predictive information regarding formulation robustness during storage and use, particularly in the initial stage of formulation development.

**Animals**

Healthy male Wistar rats (8–10 weeks old, 100–150 g) were acclimatized for seven days under controlled laboratory conditions (22 ± 2 °C; 50–60% humidity; 12-h light/dark cycle) with free access to food and water. Only animals with intact skin at the application site and no clinical signs of disease were included in the study. Rats with dermatitis, body weight outside the specified range, or signs of systemic illness were excluded to ensure homogeneity among experimental groups and adherence to welfare standards. After acclimatization, animals were randomly assigned to experimental groups using a simple randomization method to minimize selection bias. Randomization was performed by assigning animals to groups based on a random number sequence generated prior to the experiment. Each group consisted of 5 animals and received one of the following treatments: negative control, positive control, and emulgel formulations containing different emulsifier ratios. This procedure ensured equal probability of group allocation and balanced baseline characteristics among groups.

To reduce observational bias, the in vivo anti-inflammatory assessment was conducted using a blinded evaluation approach. The investigator responsible for measuring paw edema was unaware of group allocation and treatment identity during data collection. Formulations were coded by an independent laboratory member not involved in the evaluation process, and the code was revealed only after completion of data analysis. This blinding strategy was applied to all experimental groups to ensure objective measurement of outcomes. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Universitas Muhammadiyah Malang with number E.5.a/022/KEPKUMM/II/2025 and complied with OECD and ARRIVE guidelines for animal research (Kilkenny et al., 2010; OECD, 2002).

**Anti-inflammatory Evaluation**

The anti-inflammatory activity of the emulgel formulations was evaluated using the carrageenan-induced paw edema model in rats, which is a well-established method for assessing acute inflammatory responses mediated by both early and late inflammatory phases. In this study, each experimental group consisted of 5 animals, a sample size selected based on previous topical anti-inflammatory studies using similar models, where 5 animals per group were sufficient to detect biologically meaningful differences while adhering to the principle of reduction in animal use (Daud & Suryanti, 2017; Rusli et al.,

2021). This approach ensured ethical use of animals while maintaining adequate sensitivity for comparative evaluation among formulations.

Carrageenan (1% w/v) was injected subplantarly into the right hind paw of each rat to induce acute inflammation. The emulgel formulations were applied topically at a fixed dose to the inflamed area immediately after carrageenan injection. Paw volume was measured using a plethysmometer, and the percentage of edema inhibition was calculated relative to the negative control group. All procedures were conducted under standardized conditions to ensure reproducibility.

Measurement of paw edema was performed at the 6-hour time point, which corresponds to the late phase of carrageenan-induced inflammation dominated by prostaglandin release and cyclooxygenase activity. This phase is considered particularly relevant for evaluating anti-inflammatory agents with NSAID-like mechanisms, including plant-derived flavonoids (Semis et al., 2021). Therefore, the 6-hour endpoint was selected as a biologically relevant time point to compare formulation performance while minimizing repeated handling stress to the animals.

## RESULT AND DISCUSSION

As shown in table 2, variations in Tween 80 and Span 80 ratios resulted in distinct physicochemical profiles among the three emulgel formulations. Although statistical differences were observed, the interpretation of these results should emphasize their relevance to topical performance rather than numerical variation alone. For topical emulgels, viscosity, spreadability, adhesion, and pH collectively determine ease of application, residence time on the skin, and patient acceptability. Formula F2 demonstrated a moderate viscosity both before and after the cycling test, indicating sufficient structural integrity without excessive rigidity. This viscosity range is particularly important for topical delivery, as overly viscous formulations may limit spreadability, while low-viscosity systems may reduce skin residence time and dose uniformity. In this context, the relatively smaller viscosity change observed in F2 after thermal cycling suggests better resistance to physical stress and more predictable performance during storage and use. All data results was analyzed statistically using One Way Anova (α=0.05) Physicochemical parameters such as viscosity, become increase after cycling test.

**Table 2.** Physicochemical result of emulgel loaded *Calotropis gigantea* leaves extract

| Condition | Viscosity (cps) | | | Spreadabilty (cm) | | | Stickiness (sec) | | | pH | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F1 | F2 | F3 | F1 | F2 | F3 | F1 | F2 | F3 |
| Pre-cycling test | 2500 ± 134.91 | 1118 ± 0.00 | 550 ± 51.00 | 10.13 ± 0.09 | 13.07 ± 0.05 | 12.20 ± 0.10 | 2.7 ± 0.47 | 2.7 ± 0.47 | 2.3 ± 0.47 | 5.79 ± 0.01 | 5.81 ± 0.01 | 5.78 ± 0.01 |
| Post-cycling test | 3020 ± 180.55 | 1430 ± 74.83 | 960 ± 345.54 | 9.867 ± 0.05 | 10.47± 0.05 | 11.17 ± 0.05 | 2.3 ± 0.47 | 2.3 ± 0.47 | 1.3 ± 0.47 | 5.77 ± 0.00 | 5.79 ± 0.01 | 5.70 ± 0.00 |

### Viscosity

As shown in table 2, after cycling tests, viscosity values for F1, F2, and F3 increased to 20.8%, 27.9%, and 74.5%, respectively. The results indicate that formula with high Tween 80 concentration ratio can increase viscosity of formed emulgel. The emulgel formulas are all thick and green. *Calotropis gigantea* leaves extract's dark green appearance

represents what provides the emulgel the appearance of being green. The extract's green color results from the presence of green components like chlorophyll in the leaves that are removed during the maceration process, which allows the compound to dissolve in organic solvents like ethanol (Ahmad, 2020). HPMC is a gelling agent that is responsible for the thick mass that forms. The increase in viscosity observed after cycling tests can be attributed to temperature-induced rearrangement of the HPMC polymer network within the emulgel matrix. Exposure to elevated temperatures enhances polymer chain mobility, while subsequent cooling promotes chain entanglement and increased intermolecular interactions, resulting in higher apparent viscosity (Houhat et al., 2025).

This phenomenon reflects physical restructuring rather than chemical degradation of formulation components. However, because of variations in the emulsifier ratio, the viscosity varies throughout formulations. The amount of HPMC used as a gelling agent is the same in all formulations, however their viscosities varied. This is because of the disparity in the ratio of emulsifier concentration. Increased viscosity is the result of adding extra Tween 80. An interfacial film complex will be formed when the hydrophilic groups in Tween 80 bind to the flavonoid structure, which contains many hydroxyl groups (Chen et al., 2017). When reacting with oil globules, Tween 80's molecular weight is also higher than Span 80's, which results in high energy activation (Wahyuni et al., 2020). Additional studies have shown that increasing the amount of Tween 80 in an emulsion can increase its viscosity (Purwanto et al., 2023).

**Spreadability and Stickiness**

According to table 2, the spreadability values of F1, F2 and F3 decreased 2.6%; 24.8%; and 9.2%, respectively, following cycling test across all formulations. Although the trend was consistent, the difference was not statistically significant ($p > 0.05$), indicating that spreadability might be less sensitive to thermal stress than viscosity. All formulas' values of spreadibility and stickiness, meanwhile, do not differ statistically significantly. This is due to the fact that the type and concentration of gelling agent have a significant impact on the spreadability value (Milutinov et al., 2023). Gelling agents of the same type and concentration are used in all emulgel preparations to ensure that the spreadability value is not greatly impacted. Stickiness levels are lower in formulas with lower Tween 80 concentrations and vice versa. The interaction between HPMC as a gelling agent and Tween 80 is not significant despite the fact that the stickiness value changes when the concentration of Tween 80 in the emulgel increases (Pawestri & Sulaiman, 2019). The type and concentration of gelling agent employed affect stickiness (Khafifa et al., 2022).

**pH**

The pH values of each formulation to be similar statistically, so pH of all emulgel formula was similar aproximately. When compared to the emulsifier, the pH of the giant milkweed leaves extract emulgel indicates that it is within the 4.1-5.8 range needed for topical applications (Proksch, 2018). This is due to adding pH 6 buffer solution affects the pH value. This results showed that the buffer can maintain pH of emulgel.

**Cycling Test**

All emulgel physicochemical characteristic were changed. The spreadabilty, stickniness and pH of all emulgel formula were decrease. It is indicated that extreme changing temperature can affect this parameter. However, the viscosity of all emulgel formula was increase. Following the cycling test (Figure 1), the evaluation test's viscosity test results increased. All formulations showed an increase in viscosity after the cycling test, indicating that temperature variation affected the physical structure of the emulgel. However, the extent of this change differed among formulations.

F3 exhibited the largest increase in viscosity, suggesting lower resistance to thermal stress. In contrast, F2 showed a smaller and more controlled change, indicating better physical stability. This result suggests that a balanced Tween 80 and Span 80 ratio (5:5) contributes to a more stable emulsion–gel system. From a pharmaceutical perspective, better physical stability is important to prevent excessive changes in texture, which may affect product quality and consistency during storage. This is due to the fact that the viscosity of the emulgel is caused by the gelling component HPMC.

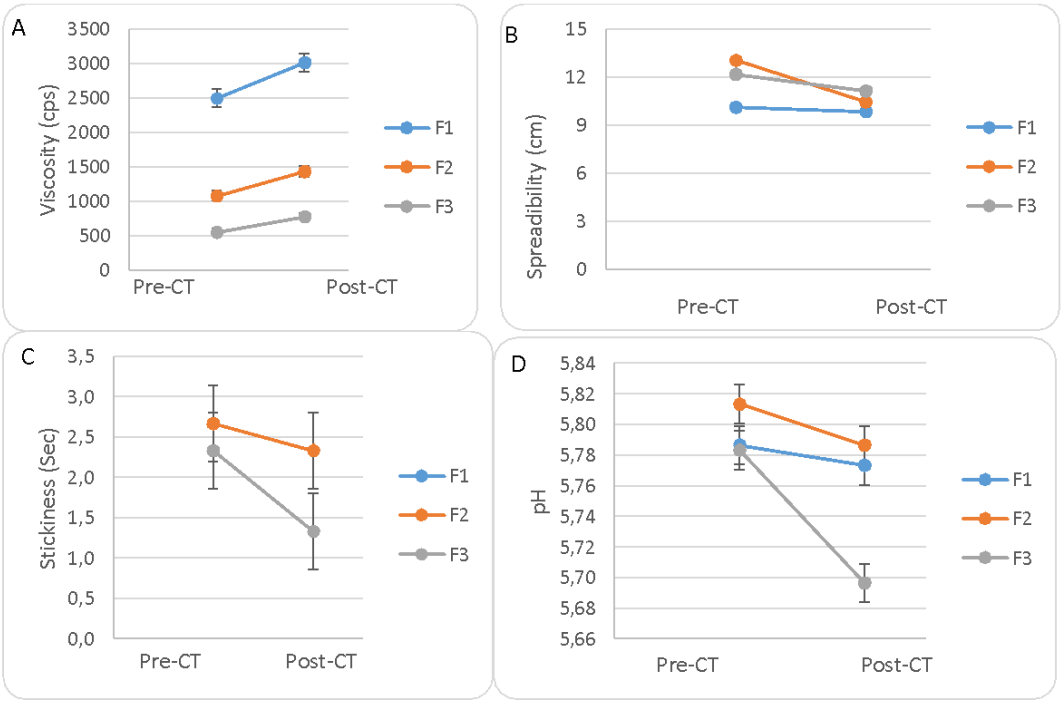



**Figure 1.** Cycling test effect to viscosity (A), spreadability (B), stickiness (C) and pH (D) of emulgel loaded *Calotropis gigantea* extract. $p<0.05$

Emulgel was exposed to both high and cooling temperatures throughout the cycling test. Because the solvent viscosity, inter-chain liaisons, and polymer solubility all diminish with increasing temperature, the polymer's molecular movements become easier (Al-Shammari et al., 2011). The viscosity of the polymer increased when the emulgel was exposed to a cooling step, which in turn raised the viscosity of the emulgel (Houhat et al., 2025). The viscosity values obtained in this study (approximately 1,250–1,700 cP) fall within the commonly reported range for topical emulgel formulations intended for anti-inflammatory application. Previous studies on herbal and NSAID-loaded emulgels have reported viscosity values ranging from 1,000 to 3,000 cP, depending on polymer type and concentration (Milutinov et al., 2023; Pawestri & Teuku Nanda, 2019). Compared to these reports, the viscosity of the optimized formulation (F2) in the present study can be considered moderate and comparable, suggesting a balance between adequate structural integrity and acceptable spreadability for topical administration.

After the cycling test, spreadability and stickiness values of all formulations decreased. This trend is consistent with the observed increase in viscosity. However, these changes were not statistically significant and remained within acceptable ranges for topical preparations. Among all formulations, F2 maintained a balance between spreadability and stickiness, indicating that it can be easily applied while still adhering adequately to the skin

surface. Higher viscosity restricts lateral spreading and reduces surface contact dynamics between the emulgel and substrate, which is commonly reported in polymer-based topical formulations subjected to thermal stress (Khafifa et al., 2022; Milutinov et al., 2023). This is because varying temperatures during storage frequently increase the viscosity of the emulgel. This means that as viscosity increases, spreadability will also decrease. As a result, the adhesion test results declined. At the same time, the adhesion test dropped.

The decreased adhesion following the cycling test is a result of quality changes brought on by temperature and pressure variations during storage. These characteristics are important for topical products, as good spreadability facilitates uniform application, while sufficient stickiness helps maintain contact with the skin for a longer period. Therefore, the physicochemical profile of F2 supports its practical suitability as a topical emulgel. Spreadability values obtained in this study remained within 5–7 cm, which is consistent with the optimal range reported for emulgel systems designed for comfortable topical application (Milutinov et al., 2023; Pawestri & Teuku Nanda, 2019). The pH values of all formulations ranged between 5.70 and 5.81, both before and after the cycling test. These values fall within the normal physiological pH range of the skin, indicating that all formulations are suitable for topical application and are unlikely to cause irritation. The use of buffer solution effectively maintained pH stability regardless of emulsifier ratio variation (Proksch, 2018). It should be acknowledged that cycling tests provide only a preliminary assessment of physical stability and do not substitute for long-term or real-time stability studies, nor do they conclusively evaluate chemical degradation pathways of active compounds (Q1A(R2): Stability Testing of New Drug Substances and Products, 2003).

**Anti-inflammatory Evaluation**

The anti-inflammatory evaluation showed that all emulgel formulations containing *Calotropis gigantea* leaves extract were able to reduce carrageenan-induced paw edema compared with the negative control (table 3). Formula F2 exhibited the highest percentage of edema reduction, although the differences among formulations were relatively small. Therefore, the anti-inflammatory results should not be interpreted as a major difference in pharmacological potency, but rather as an indication that formulation characteristics may influence the effectiveness of topical delivery. The balanced emulsifier ratio in F2 likely supports better physical stability and consistency, which may contribute to more effective contact between the formulation and the skin. *Calotropis gigantea* leaves contain secondary metabolites such as flavonoid which can acting like anti-inflammatory (Alafnan et al., 2021). Flavonoid have some hidroxyl groups which may interact with Tween 80 molecules. it may cause increase solubility of flavonoid in emulgel (Chebil et al., 2007; Lv et al., 2014). However, flavonoid have low solubility in water. Thus, the concentration of Tween 80 and Span 80 should be balanced.

**Table 3.** Anti-inflammatory evaluation result of emulgel loaded *Calotropis gigantea* extract

| Group | Percentage of reduction paw rat edema (%) |
|---|---|
| Possitive control | 71.17 ± 7.50 |
| Negative control | 22.02 ± 5.65 |
| F1 | 67.12 ± 12.9 |
| F2 | 68.98 ± 1.64 |
| F3 | 59.73 ± 0.98 |

According to (Putri & Ariyanto, 2022), HLB with balance value can increase stability and improve physicochemical characterizaton of cream. In addition, increasing solubility of bioactive using surfactant like Tween 80 and Span 80, can improve bioactive release from the dosage form through the rat skin (Rahman et al., 2023). Flavonoid inhibit the secretion of pro-antiinflammatory mediators and signaling pathways, such as cytokines responsible for inflammation and NF-kB signaling pathways (Zawawi et al., 2025). On the other hand, Non-ionic surfactants such as Tween 80 are known to interact reversibly with stratum corneum lipids, increasing lipid fluidity and enhancing transdermal permeation without causing significant skin irritation. However, excessive surfactant content may disrupt barrier integrity and reduce formulation residence time.

The balanced Tween 80–Span 80 ratio in F2 likely provides sufficient penetration enhancement while maintaining skin compatibility, thereby facilitating deeper penetration of anti-inflammatory constituents into inflamed tissue (Williams & Barry, 2004). In the carrageenan-induced paw edema model, the optimized emulgel formulation (F2) demonstrated a maximum edema inhibition of approximately 68%, which is comparable to or slightly higher than the inhibition values reported for other herbal emulgel formulations, typically ranging between 45–60% (Burki et al., 2020; Khan et al., 2022). Although synthetic NSAID-based emulgels may achieve higher inhibition levels (>70%), the anti-inflammatory efficacy observed in this study is notable considering the plant-based nature of the active extract, supporting its potential as a safer topical alternative.

The results of this study indicate that the emulsifier ratio mainly affects the physical stability and performance of the emulgel, which may indirectly influence its anti-inflammatory activity. Formula F2 demonstrated a favorable combination of moderate viscosity, acceptable spreadability, stable pH, and consistent anti-inflammatory effect. However, it should be emphasized that the proposed mechanisms related to flavonoid activity and penetration enhancement are based on literature references rather than direct experimental evidence from this study. Therefore, conclusions are drawn based on observed formulation performance and in vivo outcomes, without overinterpreting the underlying mechanisms.

## CONCLUSION

This study demonstrates that variation in the Tween 80 and Span 80 ratio influences the physicochemical characteristics, physical stability, and anti-inflammatory performance of emulgel loaded with *Calotropis gigantea* leaves extract. Among the tested formulations, the emulgel containing a balanced emulsifier ratio (Tween 80:Span 80 = 5:5) showed more consistent physicochemical properties after the cycling test and produced a slightly higher reduction of carrageenan-induced paw edema compared with the other formulations. These findings indicate that the emulsifier ratio primarily affects formulation stability and performance rather than causing substantial differences in pharmacological potency. The observed anti-inflammatory effect supports the potential of *Calotropis gigantea* leaves extract to be formulated into a topical emulgel; however, this potential should be interpreted within the scope of the current study.

Several limitations must be emphasized. The stability evaluation was limited to short-term accelerated conditions and did not include long-term or real-time stability testing. In addition, quantitative phytochemical analysis and dermal irritation or skin safety assessments were not performed, which restricts conclusions regarding formulation consistency, safety, and broader applicability. Furthermore, mechanistic explanations

related to enhanced penetration or flavonoid activity were not directly investigated. Therefore, while the results suggest that the optimized emulgel formulation has promising physicochemical stability and anti-inflammatory activity, further studies are required. Future work should include extended stability studies, comprehensive skin compatibility evaluations, and more detailed analysis of active compound content to better support its development as a topical anti-inflammatory preparation.

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**AUTHOR CONTRIBUTION**

FR: Concepts or ideas; design; definition of intellectual content; literature search; experimental studies; data analysis; manuscript preparation.
AM: Concepts or Ideas; definition of intellectual content; literature search; experimental studies.
NH: Concepts or Ideas; definition of intellectual content; literature search; experimental studies.
AMU: Manuscript editing; manuscript review.
LL: Definition of intellectual content; literature search.

**ETHICS APPROVAL**

All procedures in vivo test were approved by the Institutional Animal Care and Use Committee (IACUC) of Universitas Muhammadiyah Malang with number E.5.a/022/KEPKUMM/II/2025.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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