



Formulation and Evaluation of the Physical Stability of an Anti-Inflammatory Gel Preparation Containing a Standardized Extract of *Jatropha curcas* (Duduk) Leaves with a Carbomer Base

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ABSTRACT

Jatropha curcas L. (locally known as “duduk” in East Nusa Tenggara, Indonesia) has traditionally been used as an anti-inflammatory agent. This study aimed to formulate and evaluate the physical stability and anti-inflammatory activity of a topical gel containing standardized ethanolic leaf extract of *J. curcas* using carbomer 940. The extract was obtained by maceration with 96% ethanol and standardized based on total flavonoid and phenolic contents. Three formulations containing 5% extract and 0.5%, 1.0%, and 1.5% carbomer 940 (F1–F3) were evaluated for organoleptic properties, homogeneity, pH, spreadability, viscosity, adhesion, and accelerated stability over 28 days. All formulations were homogeneous with a pale green color and mild odor. F2 (1.0% carbomer) showed optimal characteristics, with a pH of 6.82, viscosity of 4,850 cPs, spreadability of 6.21 cm, and adhesion of 14.3 s, and remained stable throughout testing. F2 also exhibited anti-inflammatory activity in the BSA protein denaturation assay, with an IC_{50} of 87.4 μ g/mL. The 1.0% carbomer 940 formulation demonstrated optimal physical characteristics, stability, and in vitro anti-inflammatory activity, indicating its potential as a natural topical anti-inflammatory preparation.

Keywords: *Jatropha Curcas*, Gel Formulation, Carbomer 940, Physical Stability, Anti-Inflammatory, Protein Denaturation, East Nusa Tenggara

INTRODUCTION

Inflammation is a fundamental physiological response of living tissues to injury, infection, or irritation, involving complex cellular and molecular interactions that ultimately serve to eliminate injurious stimuli and initiate the healing process (Chen et al., 2018). However, chronic or dysregulated inflammation underlies the pathophysiology of numerous prevalent diseases, including rheumatoid arthritis, inflammatory bowel disease, cardiovascular disorders, and neurodegenerative conditions (Furman et al., 2019). Topical anti-inflammatory preparations, particularly gels, represent an important therapeutic modality for managing localized inflammatory conditions such as musculoskeletal pain, arthritis, skin inflammations, and soft tissue injuries. These formulations offer advantages including targeted drug delivery, avoidance of systemic side effects, and improved patient compliance (Hadgraft & Lane, 2016).

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ibuprofen, and ketoprofen are the most commonly used topical anti-inflammatory agents. However, their long-term use is associated with adverse effects including gastrointestinal irritation, skin sensitization, and, in some cases, nephrotoxicity and cardiovascular complications (Bacchi et al., 2012). These limitations have stimulated growing interest in plant-based alternatives with favorable safety profiles and comparable efficacy. The rich biodiversity of tropical regions, particularly the Indonesian archipelago, offers a vast repository of medicinal plants with documented anti-inflammatory properties awaiting scientific validation and pharmaceutical development (Atanasov et al., 2021).

Jatropha curcas L. (family Euphorbiaceae), commonly known as physic nut or Barbados nut and referred to locally as "duduk" in East Nusa Tenggara (NTT), Indonesia is a multipurpose plant with extensive ethnomedicinal use across tropical and subtropical regions of Africa, Asia, and Latin America (Gubitz et al., 1999). The leaves of *J. curcas* have been used in traditional medicine for treating inflammatory conditions, rheumatic pain, skin infections, wound healing, and fever (Devappa et al., 2011). Recent studies have confirmed the presence of diverse bioactive compounds in *J. curcas* leaves, including flavonoids (vitexin, isovitexin, apigenin, quercetin), phenolic acids (caffeic acid, ferulic acid), terpenoids (jatrophenone), alkaloids, and tannins (Singh et al., 2012; Oskoueian et al., 2011; Permatasari et al., 2023).

The anti-inflammatory activity of *J. curcas* leaf extracts has been attributed primarily to their flavonoid and phenolic content. Mechanistic studies have demonstrated that these compounds inhibit cyclooxygenase (COX-1 and COX-2) enzymes, reduce prostaglandin biosynthesis, suppress nuclear factor kappa B (NF- κ B) signaling, and inhibit pro-inflammatory cytokine production (IL-1 β , IL-6, TNF- α) (Gomes et al., 2020; Wahyuni et al., 2024). Additionally, inhibition of protein denaturation a surrogate marker of anti-inflammatory activity has been demonstrated for *J. curcas* extracts in several *in vitro* studies (Mizushima & Kobayashi, 1968; Kusumawati et al., 2023).

Despite the well-documented traditional use of *J. curcas* leaves as an anti-inflammatory agent in NTT, there is a significant gap in the scientific literature regarding the development and characterization of standardized pharmaceutical gel preparations from this plant source. Previous studies have focused primarily on crude extract characterization and *in vitro* or *in*



vivo pharmacological evaluation without advancing the material into a properly formulated, standardized, and stability-evaluated dosage form (Singh et al., 2012; Oskoueian et al., 2011). This gap is particularly significant given the potential for developing locally sourced, affordable anti-inflammatory products for communities in NTT, where access to conventional pharmaceuticals is limited.

Standardization of the extract ensuring consistent phytochemical content per unit dose is a prerequisite for reliable pharmacological activity and is a fundamental requirement for quality assurance of herbal medicinal products according to WHO and ICH guidelines (World Health Organization, 2011). The Indonesian Herbal Pharmacopoeia (Farmakope Herbal Indonesia, 2020) specifies quality parameters including moisture content, total ash, and minimum TFC/TPC values that serve as benchmarks for extract acceptability.

Gel formulations are among the most preferred topical dosage forms for anti-inflammatory delivery due to their high water content, ease of application, non-greasy texture, rapid spreading and absorption, and superior patient acceptability compared to ointments and creams (Ramli et al., 2021). Carbomer (polyacrylic acid) polymers, particularly carbomer 940, are widely used as gelling agents in pharmaceutical and cosmetic preparations owing to their remarkable gelling efficiency at low concentrations, broad pH compatibility, high optical clarity, and excellent rheological properties that promote skin adhesion and prolonged contact time (Weller, 2009). The concentration of carbomer is a critical formulation variable that significantly influences viscosity, spreadability, pH, and the overall physical stability of the gel system (Hao & Heng, 2003).

The physical stability of a topical gel preparation is critical to its therapeutic efficacy and shelf-life. Parameters such as pH, viscosity, spreadability, and homogeneity must remain within acceptable ranges throughout the product's intended storage period. Carbomer-based gels are known to be susceptible to viscosity changes under varying temperature conditions, making stability testing under controlled and accelerated conditions essential for formulation optimization (Bonferoni et al., 2020). The cycling test (alternating low and high temperatures) is a widely employed accelerated stability protocol designed to detect potential physical instability through repeated thermal stress (ICH Q1A(R2), 2003).

Therefore, the present study was designed to: (1) prepare a standardized ethanolic extract of *J. curcas* leaves from NTT; (2) formulate carbomer 940-based gels containing the standardized extract at three carbomer concentrations (0.5%, 1.0%, and 1.5%); (3) evaluate the physical characteristics and stability of the formulations over 28 days and under accelerated cycling test conditions; and (4) assess the *in vitro* anti-inflammatory activity of the optimum formulation. The findings are intended to provide a scientific foundation for the development of an evidence-based, locally sourced topical anti-inflammatory product for use in NTT communities.

METHODS

1. Plant Material Collection and Authentication

Fresh leaves of *Jatropha curcas* L. were collected from Kupang Regency, East Nusa Tenggara Province, Indonesia (coordinates: 10°08'S, 123°41'E; altitude: ±95 m above sea level) during the vegetative growth phase (September 2024). Botanical authentication was performed by a taxonomist at the Herbarium, Faculty of Sciences and Engineering, Universitas Nusa Cendana (Voucher No. UNC-HRB-2025-103). Only mature, healthy leaves free from visible disease or pest damage were selected. Ethical clearance for plant collection was obtained from the institutional research ethics committee (No. 024/EC-UNC/2025).

2. Preparation and Standardization of Extract

Leaves were cleaned, dried at 40°C for 48 hours to constant weight (moisture content: $6.8 \pm 0.4\%$), and ground to a coarse powder (40-mesh). Extraction was performed by maceration of 400 g powder in 4,000 mL of 96% ethanol for 3×24 hours with occasional stirring. The 96% ethanol concentration was selected based on preliminary optimization studies and previous reports indicating that this concentration yields optimal extraction of both polar and moderately polar bioactive compounds (flavonoids and phenolic acids) from *Jatropha* species, with higher efficiency than lower ethanol concentrations (Oskoueian et al., 2011). Combined filtrates were concentrated using a rotary evaporator (Buchi R-300, Switzerland) at 40°C and dried in a water bath at 50°C to obtain a viscous, dark green extract (yield: 11.23%, w/w).

Extract standardization was performed according to Indonesian Herbal Pharmacopoeia guidelines (Farmakope Herbal Indonesia, 2020):

- a. Total Flavonoid Content (TFC): Determined using the aluminum chloride colorimetric method with quercetin as standard (purity $\geq 98\%$, Sigma-Aldrich, USA; Batch No. Q4951-10G). Results expressed as mg quercetin equivalent (QE)/g extract.
- b. Total Phenolic Content (TPC): Determined using the Folin-Ciocalteu method with gallic acid as standard (purity $\geq 99\%$, Sigma-Aldrich, USA; Batch No. G7384-100G). Results expressed as mg gallic acid equivalent (GAE)/g extract.
- c. Quality parameters: Moisture content, loss on drying, total ash content, and acid-insoluble ash content were determined per Indonesian Herbal Pharmacopoeia standards.

3. Gel Formulation

Three gel formulations (F1, F2, F3) were prepared differing only in carbomer 940 concentration (0.5%, 1.0%, and 1.5% w/w), while maintaining a constant extract concentration of 5% w/w. A blank formulation (F0, without extract) was prepared as a negative control. All formulations contained triethanolamine (TEA) for pH adjustment, propylene glycol as humectant, methylparaben as preservative, and purified water as vehicle. The exact composition is detailed in Table 1.



Table 1. Composition of *Jatropha curcas* Leaf Extract Gel Formulations

Ingredient	Function	F0 (% w/w)	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)
<i>J. curcas</i> leaf extract	Active ingredient	0.00	5.00	5.00	5.00
Carbomer 940	Gelling agent	1.00	0.50	1.00	1.50
Triethanolamine (TEA)	pH adjuster	0.30	0.32	0.28	0.25
Propylene glycol	Humectant / co- solvent	15.00	15.00	15.00	15.00
Methylparaben	Preservative	0.18	0.18	0.18	0.18
Purified water	Vehicle	<i>ad</i> 100	<i>ad</i> 100	<i>ad</i> 100	<i>ad</i> 100

F0 = blank gel (negative control); F1 = 0.5% carbomer; F2 = 1.0% carbomer; F3 = 1.5% carbomer; q.s. = quantum sufficit (to adjust pH to 6.5–7.0); *ad* = add to make up to. TEA amounts shown are actual percentages used to achieve target pH.

Preparation Procedure:

- Carbomer 940 was dispersed in 80% of the calculated volume of purified water and allowed to hydrate for 24 hours at room temperature (25–27°C) to form a uniform dispersion.
- The extract was dissolved in propylene glycol with gentle stirring (300 rpm, 15 minutes).
- Methylparaben was dissolved in a small volume of hot purified water (60°C) and cooled to room temperature.
- All components were combined with the carbomer dispersion under gentle stirring (400–500 rpm, 30 minutes) using a mechanical stirrer (IKA RW 20, Germany), avoiding air entrapment by maintaining the stirrer position below the liquid surface.
- TEA was added dropwise with continuous stirring (500 rpm, 15 minutes) until the target pH (6.5–7.0) was achieved and the gel formed.
- The volume was adjusted to 100% with purified water.
- Air bubbles were removed by vacuum degassing (Buchi V-850, Switzerland) at 100 mbar for 10 minutes.
- Gels were stored in sealed amber glass jars at room temperature (25 ± 2°C) prior to evaluation.

4. Physical Evaluation of Gel Formulations

a. Organoleptic Evaluation

Color, odor, and texture of each formulation were assessed visually and sensorially by three trained observers on days 0, 7, 14, 21, and 28.

b. Homogeneity

A small amount of gel was placed between two glass slides and pressed. Homogeneity was assessed by the absence of visible aggregates or undispersed particles under visual inspection at ×10 magnification.

c. pH Measurement

The pH of each formulation was measured using a calibrated digital pH meter (Hanna Instruments HI 2210, Romania) at 25°C on days 0, 7, 14, 21, and 28. Each measurement was performed in triplicate. Acceptable pH range for topical gel: 4.5–8.0 (Draelos & Thaman, 2006).

d. Viscosity Measurement

Apparent viscosity was measured using a Brookfield rotational viscometer (Model DV-II+Pro, USA) with spindle No. 64 at 10 rpm, 25°C on days 0, 7, 14, 21, and 28. Measurements were conducted in triplicate.

e. Spreadability Test

A fixed amount of gel (1.0 g) was placed at the center of a glass plate and covered with a second glass plate of known weight (100 g). After 1 minute, the diameter of the spread gel was measured in two perpendicular directions. Additional weights (50, 100, 150 g) were added sequentially. Spreadability at 100 g total weight was recorded as the primary measurement.

f. Adhesion Test

One gram of gel was placed on a glass plate and sandwiched with a second glass plate. A load of 1 kg was applied for 5 minutes. The plates were then separated by applying a standard force (80 g weight), and the time (seconds) required for the plates to separate was recorded as adhesion time.

g. Syneresis

Five grams of gel in a graduated centrifuge tube was centrifuged at 3,800 rpm for 5 hours. Percentage syneresis was calculated as: $[(\text{weight of separated water} / \text{initial gel weight}) \times 100\%]$.

5. Accelerated Stability Testing Cycling Test

Stability was evaluated by cycling test as per ICH Q1A(R2) guidelines (modified for herbal preparations) (ICH Q1A(R2), 2003). Six cycles were performed: each cycle consisted of storage at 4°C for 24 hours (refrigerator) followed by 40°C for 24 hours (oven). Samples were evaluated for organoleptic properties, pH, viscosity, spreadability, and presence of phase separation or syneresis after each cycle.

6. In Vitro Anti-Inflammatory Activity (Protein Denaturation Inhibition Assay)

The *in vitro* anti-inflammatory activity was evaluated using the bovine serum albumin (BSA) protein denaturation inhibition method as described by Sakat et al. (2010), with modifications. Test samples (gel formulations) were prepared in phosphate-buffered saline (PBS, pH 7.4) at concentrations of 50–400 µg/mL (expressed as extract equivalent). Diclofenac sodium (50–400 µg/mL) served as the positive reference standard. Each 2 mL reaction mixture contained 1% w/v BSA and the test sample or standard. Samples were heated at 72°C for 10 minutes and cooled.



Turbidity was measured spectrophotometrically at 660 nm. % Inhibition of denaturation = $[(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \times 100\%$. The IC_{50} value (concentration providing 50% inhibition) was determined by nonlinear regression using GraphPad Prism 9.0.

7. Statistical Analysis

All data are expressed as mean $\pm$ standard deviation (SD; $n=3$). Normality of data distribution was confirmed using the Shapiro-Wilk test ($p > 0.05$). Homogeneity of variances was confirmed using Levene's test ($p > 0.05$). Statistical analysis was performed using one-way ANOVA with Tukey's HSD post-hoc test for inter-formulation comparisons. Repeated measures ANOVA (within-subject factor: time; between-subject factor: formulation) was used for time-dependent stability data. Significance was set at $p < 0.05$. SPSS version 26.0 (IBM Corp., USA) was used for all analyses.

RESULTS

1. Extract Standardization

The standardized ethanolic extract of *J. curcas* leaves yielded 11.23% (w/w) with the following quality parameters: moisture content $6.8 \pm 0.4\%$, loss on drying $7.2 \pm 0.3\%$, total ash content $8.1 \pm 0.5\%$, acid-insoluble ash $1.3 \pm 0.2\%$. The TFC was determined to be 142.6 ± 4.8 mg QE/g extract, and the TPC was 198.4 ± 6.2 mg GAE/g extract. These values were used to calculate the expected flavonoid and phenolic content per unit dose in each formulation (Table 2).

Table 2. Standardization Parameters of *Jatropha curcas* Leaf Extract

Parameter	Result	Specification (FHI, 2020)	Compliance
Extraction yield (% w/w)	11.23 ± 0.48	$\geq 8.0\%$	✓ Pass
Moisture content (%)	6.8 ± 0.4	$\leq 10.0\%$	✓ Pass
Loss on drying (%)	7.2 ± 0.3	$\leq 10.0\%$	✓ Pass
Total ash content (%)	8.1 ± 0.5	$\leq 12.0\%$	✓ Pass
Acid-insoluble ash (%)	1.3 ± 0.2	$\leq 2.0\%$	✓ Pass
Total Flavonoid Content (mg QE/g)	142.6 ± 4.8	≥ 100 mg QE/g	✓ Pass
Total Phenolic Content (mg GAE/g)	198.4 ± 6.2	≥ 150 mg GAE/g	✓ Pass

FHI = Farmakope Herbal Indonesia; QE = Quercetin Equivalent; GAE = Gallic Acid Equivalent; Data expressed as mean $\pm$ SD ($n=3$).

2. Organoleptic Properties and Homogeneity

All gel formulations (F0–F3) were homogeneous with no visible aggregates or phase separation observed under visual and microscopic examination throughout the 28-day evaluation period. F0 (blank) was colorless to pale white; F1, F2, and F3 presented a characteristic pale-to-medium green color derived from the extract, with a mild, slightly herbaceous odor. The intensity

of green coloration increased with extract concentration, but remained consistent within each formulation over time. Texture observations revealed that gel consistency increased from F1 to F3 commensurate with increasing carbomer concentration. No phase separation, cracking, or syneresis was observed in any formulation after the cycling test.

3. pH Values

The pH values of all formulations remained within the acceptable range for topical preparations (4.5–8.0) throughout the 28-day study period (Table 3). F2 exhibited the most stable and skin-compatible pH, with minimal fluctuation across time points.

Table 3. pH Values of Gel Formulations Over 28 Days (Mean $\pm$ SD, n=3)

Formulation	Day 0	Day 7	Day 14	Day 21	Day 28	$\Delta\Delta\Delta$ vs Day 0 (Day 28)
F0 (blank)	6.91 $\pm$ 0.03	6.88 $\pm$ 0.04	6.86 $\pm$ 0.03	6.84 $\pm$ 0.04	6.83 $\pm$ 0.03	−0.08 (NS)
F1 (0.5% CB)	6.63 $\pm$ 0.04	6.57 $\pm$ 0.05	6.52 $\pm$ 0.04	6.48 $\pm$ 0.05	6.44 $\pm$ 0.06	−0.19*
F2 (1.0% CB)	6.82 $\pm$ 0.05	6.80 $\pm$ 0.04	6.78 $\pm$ 0.05	6.76 $\pm$ 0.04	6.74 $\pm$ 0.05	−0.08 (NS)
F3 (1.5% CB)	6.94 $\pm$ 0.04	6.92 $\pm$ 0.03	6.90 $\pm$ 0.04	6.88 $\pm$ 0.03	6.85 $\pm$ 0.04	−0.09 (NS)

CB = Carbomer 940; NS = Not significant ($p > 0.05$); * = Significant decrease ($p < 0.05$) vs Day 0; Tukey's HSD test.

4. Viscosity

Viscosity increased proportionally with increasing carbomer concentration. F2 (1.0% carbomer) demonstrated optimal viscosity values consistent with semisolid topical preparations (3,000–6,000 cPs) throughout the storage period. F1 showed relatively low viscosity, potentially limiting its adherence to skin surfaces, while F3 showed high viscosity that may impair spreadability. Results are summarized in Table 4.

Table 4. Viscosity Values (cPs) of Gel Formulations Over 28 Days (Mean $\pm$ SD, n=3)

Formulation	Day 0	Day 7	Day 14	Day 21	Day 28
F0 (blank)	4,680 $\pm$ 115	4,650 $\pm$ 120	4,620 $\pm$ 118	4,600 $\pm$ 125	4,575 $\pm$ 122
F1 (0.5% CB)	1,820 $\pm$ 85	1,795 $\pm$ 90	1,770 $\pm$ 88	1,748 $\pm$ 92	1,720 $\pm$ 95
F2 (1.0% CB)	4,850 $\pm$ 120	4,820 $\pm$ 118	4,795 $\pm$ 122	4,768 $\pm$ 115	4,740 $\pm$ 128
F3 (1.5% CB)	8,340 $\pm$ 145	8,280 $\pm$ 150	8,225 $\pm$ 148	8,170 $\pm$ 152	8,110 $\pm$ 155

Acceptable viscosity range for topical gel: 2,000–6,000 cPs. CB = Carbomer 940. Values in bold indicate formulations within acceptable range throughout the study.

5. Spreadability

Spreadability was inversely related to carbomer concentration. F1 exhibited the highest spreadability but at the cost of lower viscosity, whereas F3 showed the lowest spreadability. F2



demonstrated the best balance between spreadability and viscosity, consistent with optimal topical application performance. Table 5 presents the spreadability data.

Table 5. Spreadability (cm) of Gel Formulations Over 28 Days (Mean $\pm$ SD, n=3)

Formulation	Day 0	Day 7	Day 14	Day 21	Day 28
F0 (blank)	6.04 $\pm$ 0.15	6.00 $\pm$ 0.16	5.96 $\pm$ 0.14	5.92 $\pm$ 0.17	5.88 $\pm$ 0.16
F1 (0.5% CB)	7.45 $\pm$ 0.21	7.38 $\pm$ 0.19	7.31 $\pm$ 0.22	7.24 $\pm$ 0.20	7.18 $\pm$ 0.23
F2 (1.0% CB)	6.21 $\pm$ 0.18	6.17 $\pm$ 0.17	6.12 $\pm$ 0.19	6.08 $\pm$ 0.16	6.03 $\pm$ 0.18
F3 (1.5% CB)	4.38 $\pm$ 0.14	4.33 $\pm$ 0.15	4.29 $\pm$ 0.13	4.25 $\pm$ 0.14	4.21 $\pm$ 0.15

Acceptable spreadability range: 5–7 cm. CB = Carbomer 940.

6. Adhesion Time

Adhesion time was proportional to carbomer concentration. F2 showed acceptable and consistent adhesion time throughout the study, indicating adequate contact time on the skin surface for drug permeation. Table 6 presents the adhesion time data.

Table 6. Adhesion Time (seconds) of Gel Formulations Over 28 Days (Mean $\pm$ SD, n=3)

Formulation	Day 0	Day 7	Day 14	Day 21	Day 28
F0 (blank)	13.1 $\pm$ 0.8	12.9 $\pm$ 0.7	12.7 $\pm$ 0.9	12.6 $\pm$ 0.8	12.4 $\pm$ 0.9
F1 (0.5% CB)	8.2 $\pm$ 0.6	8.0 $\pm$ 0.7	7.9 $\pm$ 0.6	7.7 $\pm$ 0.7	7.5 $\pm$ 0.8
F2 (1.0% CB)	14.3 $\pm$ 0.9	14.0 $\pm$ 0.8	13.8 $\pm$ 0.9	13.5 $\pm$ 1.0	13.2 $\pm$ 0.9
F3 (1.5% CB)	22.6 $\pm$ 1.2	22.1 $\pm$ 1.1	21.7 $\pm$ 1.3	21.2 $\pm$ 1.2	20.8 $\pm$ 1.4

Acceptable adhesion time: ≥ 4 seconds. CB = Carbomer 940.

7. Accelerated Stability Testing (Cycling Test)

All formulations retained their homogeneity and characteristic properties after 6 cycling test cycles (Table 7). No phase separation or syneresis was observed in F2 and F3. F1 showed minimal syneresis (0.42 $\pm$ 0.08%) in cycle 4–6, indicating slightly reduced physical stability at lower carbomer concentration. pH and viscosity changes in F2 after cycling were within $\pm 5\%$ of initial values, meeting the criterion for stability acceptance.

Table 7. Summary of Physical Characteristics After Cycling Test (6 Cycles) (Mean $\pm$ SD, n=3)

Parameter	F0	F1	F2	F3	Acceptance Criteria
Color	Colorless	Pale green	Pale green	Medium green	No significant change
Odor	Odorless	Mild herbal	Mild herbal	Mild herbal	No significant change

Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	No aggregates
pH	6.81 ± 0.04	6.40 ± 0.07	6.72 ± 0.05	6.83 ± 0.04	4.5–8.0
Viscosity (cPs)	4,540 ± 130	1,695 ± 98	4,710 ± 132	8,050 ± 160	Within ±10% of Day 0
Spreadability (cm)	5.85 ± 0.17	7.12 ± 0.25	6.00 ± 0.20	4.18 ± 0.16	5–7 cm
Syneresis (%)	0.00	0.42 ± 0.08	0.00	0.00	< 1.0%
Phase separation	None	None	None	None	None

All values post-cycling test. CB = Carbomer 940.

8. In Vitro Anti-Inflammatory Activity

The *in vitro* anti-inflammatory activity evaluated by the BSA protein denaturation inhibition assay showed that all gel formulations containing extract produced concentration-dependent inhibition (Table 8). F2 exhibited the highest anti-inflammatory activity among the gel formulations, with an IC_{50} of $87.4 \pm 2.3 \mu\text{g/mL}$. The anti-inflammatory potency order was: Diclofenac sodium > F2 > F3 > F1. The IC_{50} of F2 ($87.4 \pm 2.3 \mu\text{g/mL}$) was approximately 2.08-fold less potent than diclofenac sodium ($42.1 \pm 1.8 \mu\text{g/mL}$).

Interestingly, F2 showed higher activity than F3 despite both containing equal amounts of extract (5%), suggesting that the rheological properties of F2 may better facilitate release of the active compounds into the assay medium. This finding is consistent with optimal dissolution characteristics at intermediate carbomer concentrations, where the polymer network density allows optimal diffusion of flavonoid and phenolic compounds compared to the denser and more tortuous diffusion path in F3.

Table 8. In Vitro Anti-Inflammatory Activity (% Inhibition of BSA Denaturation) and IC_{50}

Values				
Concentration ($\mu\text{g/mL}$)	Diclofenac sodium (% inh.)	F1 (% inh.)	F2 (% inh.)	F3 (% inh.)
50	38.2 ± 1.4	21.5 ± 1.2	28.4 ± 1.3	25.8 ± 1.1
100	56.8 ± 1.6	36.2 ± 1.5	47.6 ± 1.6	42.3 ± 1.4
200	74.3 ± 1.8	56.4 ± 1.7	64.8 ± 1.9	59.7 ± 1.6
400	88.6 ± 2.1	72.1 ± 2.0	79.3 ± 2.1	74.5 ± 1.9
IC_{50} ($\mu\text{g/mL}$)	42.1 ± 1.8	112.6 ± 3.1	87.4 ± 2.3	98.2 ± 2.7

% inh. = percentage inhibition of BSA denaturation. IC_{50} = concentration producing 50% inhibition. Data expressed as mean ± SD (n=3). Lower IC_{50} indicates higher anti-inflammatory potency.

The anti-inflammatory potency order was: Diclofenac sodium > F2 > F3 > F1. The IC_{50} of F2 ($87.4 \pm 2.3 \mu\text{g/mL}$) was approximately 2.08-fold less potent than diclofenac sodium ($42.1 \pm 1.8 \mu\text{g/mL}$).



µg/mL). Interestingly, F2 showed higher activity than F3 despite both containing equal amounts of extract (5%), suggesting that the rheological properties of F2 may better facilitate release of the active compounds into the assay medium, a finding consistent with optimal dissolution characteristics at intermediate carbomer concentrations.

DISCUSSION

This study successfully formulated and characterized three carbomer 940-based gel preparations containing a standardized ethanolic extract of *Jatropha curcas* leaves from East Nusa Tenggara, Indonesia. The results demonstrate that formulation F2 (1.0% carbomer 940, 5% extract) represents the optimum formulation, exhibiting well-balanced physical characteristics, superior physical stability over 28 days and under accelerated cycling test conditions, and the highest *in vitro* anti-inflammatory activity among the tested gel formulations.

The extraction yield of 11.23% obtained in this study is consistent with values previously reported for *J. curcas* leaf ethanolic extracts. Singh et al. (2012) reported extraction yields of 8.5–13.2% for ethanol extracts of *J. curcas* leaves depending on extraction parameters and plant origin. The standardization parameters, including TFC (142.6 ± 4.8 mg QE/g) and TPC (198.4 ± 6.2 mg GAE/g), indicate a high content of flavonoid and phenolic compounds, which are the primary bioactive constituents responsible for anti-inflammatory activity (Permatasari et al., 2023; Wahyuni et al., 2024). These values exceed the minimum specifications established in the Indonesian Herbal Pharmacopoeia, confirming the suitability of this extract for pharmaceutical formulation (Farmakope Herbal Indonesia, 2020).

The pH values of all formulations (6.44–6.94) remained within the physiologically acceptable range for topical skin application (4.5–8.0) throughout the 28-day study. Skin pH typically ranges from 4.5 to 5.5, and gel preparations intended for topical use should ideally have a pH close to this range to minimize irritation and support skin barrier function (Draelos & Thaman, 2006). The slightly alkaline pH of carbomer-based gels is a well-known characteristic of this gelling agent system, as the neutralization with TEA generates sodium or ammonium polyacrylate salts. The observation that F1 showed a statistically significant pH decrease over time (-0.19 , $p < 0.05$) may be attributed to its lower buffering capacity at the reduced carbomer concentration, rendering it more susceptible to pH drift due to the acidic components of the extract (Ramli et al., 2021).

Viscosity is a critical quality parameter for topical gels, as it directly influences the ease of application, spreadability, and skin residence time. The optimal viscosity range for topical gels has been reported as 3,000–6,000 cPs in most pharmaceutical literature (Hao & Heng, 2003). In this study, F1 (1,720–1,820 cPs) fell below this range, indicating insufficient gel strength that may result in runoff from the application site. F3 (8,110–8,340 cPs) exceeded the upper limit, potentially making the product difficult to spread and reducing patient compliance. F2 (4,740–4,850 cPs) consistently remained within the optimal range throughout the study, confirming its formulation suitability.

The relationship between carbomer concentration and viscosity follows a power-law relationship typical of polymer solutions, where small increases in polymer concentration produce

exponential increases in viscosity. At 0.5% carbomer, the polymer chains are insufficiently entangled to form a continuous three-dimensional network capable of immobilizing the aqueous phase, resulting in low viscosity and poor structural integrity. At 1.5% carbomer, the network becomes overly dense, creating a stiff gel with limited spreadability (Ramli et al., 2021). At 1.0% carbomer, the polymer concentration is optimal for forming a well-entangled network with appropriate mechanical properties for topical application.

The gradual decrease in viscosity observed across all formulations over 28 days is consistent with the known susceptibility of carbomer gels to minor viscosity reduction during storage due to partial hydrolysis and chain relaxation of the polyacrylic acid network, and remained within acceptable limits for all formulations except F3 and partially F1 (Weller, 2009). This phenomenon is attributed to the slow deprotonation of carboxylic acid groups and rearrangement of polymer chains toward thermodynamic equilibrium.

Spreadability reflects the ease with which a semisolid preparation can be applied to the skin surface and is inversely related to viscosity. The acceptable spreadability range for topical gels (5–7 cm under standard conditions) encompasses F0 and F2 throughout the study, while F1 exceeded the upper limit (indicating a preparation that may spread excessively and reduce local drug concentration at the application site), and F3 fell below the lower limit, reflecting the high-viscosity, stiff consistency of the 1.5% carbomer system. These findings are in agreement with the general principle that intermediate carbomer concentrations provide the optimal balance between gel strength and application properties (Bonferoni et al., 2020).

The cycling test is a widely employed accelerated stability protocol for topical preparations, designed to detect potential physical instability through repeated thermal stress. The ability of F2 to maintain all physical parameters within acceptable ranges across 6 cycles (4°C/40°C) demonstrates robust formulation stability and is indicative of a thermally stable system. The minimal syneresis observed in F1 ($0.42 \pm 0.08\%$ after cycling) confirms that 0.5% carbomer is insufficient to form a three-dimensional polymer network of adequate strength to retain water under physical stress, a finding consistent with published data on carbomer gel syneresis at sub-optimal concentrations (Hao & Heng, 2003). Syneresis, if significant, would indicate instability of the gel matrix and potential phase separation upon storage, leading to inconsistent dosing and reduced shelf-life.

The *in vitro* anti-inflammatory activity assessed by BSA protein denaturation inhibition provided important insights into the pharmacological potential of the gel formulations. Protein denaturation is one of the pathophysiological mechanisms of inflammation: heat-induced denaturation of serum proteins can trigger immune responses, and the ability of a preparation to inhibit this process is a well-established surrogate marker of anti-inflammatory activity, frequently used in the evaluation of plant extracts and formulated products (Mizushima & Kobayashi, 1968). The IC_{50} of F2 ($87.4 \pm 2.3 \mu\text{g/mL}$) compares favorably with values reported for other plant-based anti-inflammatory topical preparations; for instance, Gomes et al. (2020) reported IC_{50} values of 95–180 $\mu\text{g/mL}$ for flavonoid-rich plant extract gels in the same assay.



The observation that F2 showed higher anti-inflammatory activity than F3 despite identical extract content (5%) warrants mechanistic explanation. This phenomenon can be understood through the "polymer mesh theory" of drug release from carbomer matrices. At 1.5% carbomer (F3), the polymer network is highly dense, with smaller mesh sizes (estimated pore diameter < 10 nm) that physically entrap the extract constituents, particularly the larger flavonoid glycosides (molecular weight ~400–600 Da). This entrapment reduces the diffusion coefficient of the active compounds and limits their release from the gel matrix. In contrast, at 1.0% carbomer (F2), the polymer network is less dense (estimated mesh size 15–25 nm), allowing more favorable diffusion of the bioactive compounds into the assay medium. This finding is consistent with drug release studies from carbomer matrices, where intermediate polymer concentrations provide optimal balance between network integrity and drug diffusion (Bonferoni et al., 2020; Ramli et al., 2021).

The anti-inflammatory potency of *J. curcas* extract is attributed to its flavonoid and phenolic constituents, particularly quercetin and apigenin derivatives, which have been shown to inhibit COX-2, lipoxygenase, and NF- κ B signaling pathways (Oskoueian et al., 2011; Gomes et al., 2020; Wahyuni et al., 2024). Quercetin, a major flavonoid in *J. curcas* leaves, has been extensively studied for its anti-inflammatory mechanisms, including inhibition of pro-inflammatory cytokine production, suppression of iNOS and COX-2 expression, and modulation of the NF- κ B and MAPK pathways (Permatasari et al., 2023). The phenolic acids present in the extract, including caffeic and ferulic acids, contribute synergistic antioxidant and anti-inflammatory effects (Kusumawati et al., 2023).

While the *in vitro* BSA denaturation assay provides useful screening data, it should be noted that *in vitro* results do not fully predict *in vivo* efficacy, and further pharmacokinetic, ex vivo skin permeation, and *in vivo* anti-inflammatory studies are required to fully validate the therapeutic potential of this preparation. The gel formulation developed in this study must undergo additional characterization including drug release kinetics, skin permeation studies using Franz diffusion cells, and evaluation of skin irritation potential before clinical application.

From a practical and public health perspective, the development of a stable, well-characterized topical anti-inflammatory gel from locally cultivated *J. curcas* leaves carries significant implications for healthcare access in NTT. The availability of affordable, locally produced herbal medicinal products formulated to pharmaceutical standards could substantially reduce dependence on expensive imported NSAID preparations and align with Indonesia's national policy on the utilization of local medicinal plant resources (Biofarmaka) for primary healthcare.

The development of this formulation for local production in NTT requires consideration of several practical factors. Carbomer 940, propylene glycol, and methylparaben are commercially available through pharmaceutical suppliers in major Indonesian cities (Jakarta, Surabaya, Makassar) and can be procured at reasonable cost (approximately IDR 250,000–400,000/kg for carbomer 940). TEA is widely available at lower cost (approximately IDR 100,000–150,000/kg). The extract preparation requires ethanol (96%) which is available from local distilleries in NTT at approximately



IDR 30,000–40,000/L. The use of locally sourced plant material eliminates import costs for the active ingredient, providing a significant economic advantage.

The tropical climate of NTT (temperatures 25–35°C, high humidity) poses challenges for product stability. Our accelerated cycling test results (6 cycles at 4–40°C) suggest that the optimized formulation (F2) has adequate thermal stability to withstand tropical conditions. However, for long-term storage in hot climates, packaging should include UV-protective containers (amber glass or opaque plastic tubes) and storage recommendations should advise keeping the product in cool, dry conditions. Aluminum tubes with inner lacquer coating are recommended for final packaging to prevent light and oxygen penetration.

The scale-up from laboratory to pilot production requires consideration of mixing equipment, homogenization parameters, and quality control procedures. A simple mechanical stirrer (IKA RW series or equivalent, approximately IDR 15–20 million) is sufficient for small-scale production (1–5 kg batches). Quality control testing for each batch should include pH measurement, viscosity determination, and TFC/TPC content assay to ensure batch-to-batch consistency.

The standardization approach adopted in this study anchored to TFC and TPC as chemical markers ensures batch-to-batch consistency, a fundamental requirement for herbal product quality assurance. This approach is recommended by the WHO for quality control of herbal medicinal products (World Health Organization, 2011). For future commercial development, additional marker compounds (e.g., quercetin or apigenin quantified by HPLC) could be incorporated into the specification to provide more specific chemical characterization.

CONCLUSIONS

Three carbomer 940-based gel formulations containing 5% standardized ethanolic extract of *Jatropha curcas* leaves from East Nusa Tenggara were successfully prepared and evaluated. Formulation F2, containing 1.0% carbomer 940, demonstrated optimal and well-balanced physical characteristics, including pH 6.82 ± 0.05 , viscosity $4,850 \pm 120$ cPs, spreadability 6.21 ± 0.18 cm, and adhesion time 14.3 ± 0.9 seconds. F2 maintained stable physical properties over 28 days of storage and withstood accelerated cycling test conditions without significant changes. F2 also exhibited the highest in vitro anti-inflammatory activity (IC_{50} : 87.4 ± 2.3 µg/mL) among gel formulations, compared to the standard diclofenac sodium (IC_{50} : 42.1 ± 1.8 µg/mL). These findings establish F2 as the optimum formulation and support the further development of *J. curcas* leaf extract gel as a natural, standardized topical anti-inflammatory product. Future work should encompass in vitro drug release and skin permeation studies, in vivo pharmacological validation, safety evaluation, and longer-term stability assessment under ICH conditions.

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