

Development of balsem plant (*sennega paniculata* linn.) nano spray gel as a topical analgesic: Optimization of in vivo efficacy and physical stability

Ismat Marsus¹, Muhammad Khaerul Nur², Holinda Anggrainy³, Yandiono Pongroba⁴

^{1,2,3,4}Department Pharmacy, Universitas Indonesia Timur, Makassar, Indonesia

ARTICLE INFO

Article history:

Received Aug 10, 2026

Revised Aug 22, 2026

Accepted Aug 30, 2026

Keywords:

Nano-Spray Gel
Particle Size Analysis
Preliminary in Vivo Efficacy
Sennega Paniculata L
Topical Analgesic

ABSTRACT

The development of natural topical analgesics is constrained by physical instability and limited stratum corneum permeability. Although *Senega paniculata* L. extract exhibits bioactive potential, its formulation into a calcium-carboxymethyl cellulose (Ca-CMC) nano-spray gel matrix to overcome skin permeability barriers and enhance delivery remains largely unexplored. This study aims to formulate a Ca-CMC nano-spray gel using *S. paniculata* L. extract across different concentrations (FI 5%, FII 10%, FIII 15%) and to evaluate its preliminary physicochemical properties and analgesic potential in an animal model. Formulations were assessed for stability, physicochemical properties, and chemical interactions. Particle size and polydispersity index were evaluated using Dynamic Light Scattering, while functional groups were analyzed via FTIR. Analgesic efficacy was preliminarily evaluated using an acute thermal pain model to determine the percentage of maximum possible effect. All formulations maintained physiological pH. Formulation FIII exhibited optimal characteristics with a Z-average of 94.6 nm and a polydispersity index of 0.128. FTIR spectra confirmed Ca-CMC ionic cross-linking. In the preliminary animal test, FIII demonstrated an analgesic efficacy of 70.54% ($p = 0.958$). This study provides preliminary evidence regarding formulation characteristics and preliminary analgesic activity in animals, rather than proving that the product has been fully validated as a clinically effective and stable analgesic formulation. These findings offer a foundational basis for further optimization and development of *S. paniculata* L. calcium nanocolloids as topical analgesics.

This is an open access article under the [CC BY-NC](https://creativecommons.org/licenses/by-nc/4.0/) license.



Corresponding Author:

Ismat Marsus,
Department of Pharmacy,
Universitas Indonesia Timur,
Jl. Abdul Kadir No. 70 Balang Baru Sub-district, Makassar, 90224, Indonesia
Email: ismatmarsus23@gmail.com

INTRODUCTION

This study addresses the adverse risks of long-term synthetic analgesic use and the limited application of *Senega paniculata* L., which remains largely restricted to conventional extraction despite its rich flavonoid content capable of inhibiting cyclooxygenase enzymes to relieve pain and

inflammation (Iskandar et al., 2025; Krakowska-Sieprawska et al., 2022). As the physicochemical performance and therapeutic efficacy of *S. paniculata* L. within nanostructured topical vehicles remain unexplored, this research offers novelty by being the first to integrate its 96% ethanolic extract into a nano-spray gel system (Gonfa et al., 2023; Patala et al., 2023; Rijai, 2013). Ultimately, this work aims to demonstrate that formulating the extract at concentrations of 5%, 10%, and 15% w/v yields a novel delivery vehicle exhibiting optimal physicochemical properties, robust thermal stability, favorable skin safety, and potent *in vivo* analgesic activity comparable to standard therapeutic controls (Chenna M shalini et al., 2025; Karlina et al., 2024; Nisrina et al., 2025).

RESEARCH METHOD

Study Design

This study adopted a true experimental design based on a Completely Randomized Design (CRD) to evaluate the physical stability and preliminary *in vivo* analgesic efficacy of a nano-spray gel incorporating a 96% ethanolic extract derived from the aerial parts of *Senega paniculata* Linn. (Specimen No. B-3638/II.6.2/IR.01.02/8/2025) formulated at 5% (FI), 10% (FII), and 15% (FIII) w/v, against a negative control (Na-CMC vehicle base) and a positive control (Voltaren® 1% gel). The independent variable was the *S. paniculata* extract concentration, whereas the dependent variables comprised physicochemical performance, physical stability (monitored over 28-day ambient storage and thermal cycling), and *in vivo* analgesic performance. Animal sample sizes were calculated using Federer's equation $(t-1)(n-1) \geq 15$, yielding a minimum threshold of five male mice (*Mus musculus*) across five treatment cohorts ($n = 5$, total $N = 25$), assigned via simple random allocation and observed under a single-blind protocol to mitigate evaluation bias. The procedure involved macerating pulverized aerial biomass in 96% ethanol and synthesizing calcium oxide (CaO) nanoparticles by reacting CaCl_2 and KOH precursors into Ca(OH)_2 , followed by 700°C calcination, which were subsequently ionic cross-linked into a Na-CMC hydrogel matrix under high-shear homogenization (15,000 rpm). Nanoscale formation was verified using Dynamic Light Scattering (DLS) to determine the hydrodynamic diameter (Z-average) and polydispersity index (PDI), alongside Fourier-Transform Infrared (FTIR) spectroscopy confirming cross-linking through asymmetric and symmetric COO^- carboxylate shifts and a characteristic Ca-O metal-oxide absorption peak. Thermal nociceptive latencies were measured using a hot-plate apparatus ($55.0 \pm 0.5^\circ\text{C}$) at 30, 60, 90, and 120-minute post-application intervals (utilizing a 15-second cut-off threshold), with percentage analgesic efficacy evaluated through Repeated-Measures ANOVA and Tukey's HSD post hoc testing ($p < 0.05$) (Tapfumaneyi et al., 2022).

Tools and Materials

Tools All experimental procedures, physicochemical characterizations, and *in vivo* evaluations were conducted using high-precision, calibrated analytical instrumentation: a Class II Esco Airstream® Laminar Air Flow cabinet provided HEPA-filtered aseptic conditions, while raw material gravimetric measurements were performed on a Changxie FA2004 analytical balance (precision 0.1 mg). Controlled thermal extraction was executed via a Benchmark HP-300 hotplate stirrer, and crude extracts were concentrated using a Buchi R-300 rotary evaporator under reduced pressure. Colloidal nanostructures were synthesized utilizing an IKA® T25 Digital Ultra-Turrax high-shear homogenizer (3,000-25,000 rpm), followed by particle size (Z-average) and polydispersity index (PDI) analysis via Dynamic Light Scattering (DLS) on a Malvern Zetasizer Nano ZS (0.3 nm-10 μm). Chemical structures and ionic cross-linking motifs were mapped across 4000-400 cm^{-1} using a IR Prestige-21 Shimadzu FTIR spectrometer, and formulation acidity was verified using a Hanna Instruments HI-2211 benchtop pH meter (± 0.01 pH accuracy). Finally, *in vivo* thermal nociceptive testing in animal models was quantified at $55.0 \pm 0.5^\circ\text{C}$ on a Harvard Apparatus Thermal Hot Plate coupled with a Casio HS-3V digital precision timer (0.01 s

resolution), with all general liquid transfers and macerations handled using Pyrex® borosilicate 3.3 glassware.

Materials Aerial parts of balsam plant (*Senega paniculata* Linn.) were collected and taxonomically verified prior to extraction. High-grade raw materials and pharmaceutical excipients utilized in the formulations comprised 96% ethanol (extraction solvent), sodium carboxymethyl cellulose (Na-CMC; primary hydrogel matrix), calcium chloride (CaCl_2 ; ionic cross-linker), propylene glycol (humectant and skin permeation promoter), triethanolamine (pH regulator), methylparaben (microbial preservative), peppermint oil (olfactory additive), and double-distilled water (aqueous vehicle). Commercial Voltaren® gel (1% diclofenac sodium) was procured to serve as the positive reference drug (Karlina et al., 2024; Sawu et al., 2025; WOS Zubaydah WOS Zubaydah et al., 2022).

Research Procedures

Preparation of Balsam Plant Extract Aerial parts of *Sennega paniculata* Linn. were authenticated at BRIN (Specimen No. B-3638/II.6.2/IR.01.02/8/2025) and deposited in the herbarium. Dried, ground plant material (250 g) was macerated in 96% ethanol (2000 mL, 1:8 w/v ratio) at 25 ± 2 °C with periodic agitation. The process involved five 24-hour cycles. After each cycle, the mixture was filtered through Whatman No. 1 paper. Pooled filtrates were concentrated under reduced pressure using a rotary evaporator, yielding 30 g of thick crude extract (Floyd et al., 2025).

Synthesis and Characterization of Calcium (Ca) Nanoparticles from Balsam Plant Extract Synthesis of calcium oxide (CaO) nanoparticles was initiated by digesting 100-mesh *Senega paniculata* Linn. aerial biomass in 3N HCl under constant agitation (90 rpm) to extract endogenous calcium ions as soluble CaCl_2 , which was subsequently precipitated into a $\text{Ca}(\text{OH})_2$ nanonucleation intermediate by controlled dropwise addition of 3N KOH across alkaline targets (pH 9-13) and reaction durations (30-150 min). Following neutral washing and oven-drying at 100°C, the precursor was calcined at 700°C for 2 hours to induce complete thermal dehydration into a pure cubic CaO crystalline phase ($\text{Ca}(\text{OH})_2 \rightarrow \text{CaO} + \text{H}_2\text{O}$), as confirmed by FTIR through the characteristic Ca-O metal-oxide absorption. The resulting CaO nanoparticles were ionic cross-linked with hydrogel carboxylate (COO^-) motifs and dispersed into a stable nanocolloidal spray gel system via high-shear homogenization (15,000 rpm), yielding an optimal hydrodynamic particle size (Z-average) and a narrow polydispersity index (PDI) verified via Dynamic Light Scattering (DLS) (Mishra et al., 2024; Pasiieczna-Patkowska et al., 2025).

Design and Formulation of Nano Spray Gel The preparation of the nanocolloidal spray gel commenced with hydrating sodium carboxymethyl cellulose (Na-CMC) in hot distilled water (70-80°C) to establish a uniform hydrogel matrix, into which a pre-blended mixture of propylene glycol, methylparaben, and triethanolamine was homogeneously incorporated to regulate vehicle humectancy, preservation, and physiological pH (4.5-7.0). Ethanolic extract of *Senega paniculata* Linn. (5%, 10%, or 15% w/v), calcined kalsium oksida (CaO) nanoparticles, and peppermint oil were sequentially introduced under continuous stirring, followed by high-shear homogenization (15,000 rpm) to achieve ionic cross-linking and stable nanocolloidal droplet dispersion *q.s.* 100 mL (WOS Zubaydah WOS Zubaydah et al., 2022).

Analgesic Test

Analgesic Activity Testing Procedure For the *in vivo* analgesic screening, 25 acclimatized male Swiss Webster mice (*Mus musculus*), aged 8-10 weeks (22-28 g), were randomly assigned to five cohorts (n = 5); negative control, positive control (Voltaren® 1% gel), and treatment groups FI (5%), FII (10%), and FIII (15%). Animals were sourced from Makassar City Department of Livestock and Animal Health and housed under standard conditions (22 ± 2 °C, 12-h light/dark cycle) with *ad libitum* feeding under ethical clearance (No. 800/6150/DISNAK-KESWAN). Following single topical plantar application under single-blind protocol, thermal nociceptive

latency responses were measured on a hot-plate apparatus ($55.0 \pm 0.5^{\circ}\text{C}$) at 30-min intervals over 30–120 min, applying a 15-s cut-off threshold to avoid tissue injury. Calculated percentage analgesic efficacies (%MPE) were analyzed using Repeated-Measures ANOVA with Tukey’s HSD *post hoc* test ($p < 0.05$). (Kurniasih et al., 2025; Sawu et al., 2025).

Data Analysis

Observational data were tabulated and evaluated using Repeated-Measures ANOVA following verification of normality and homogeneity assumptions. Post-hoc comparisons were conducted using the Tukey’s HSD post hoc test at a 95% confidence level ($\alpha = 0.05$). Nanoparticle characterization via FTIR spectra was evaluated descriptively. All statistical computations were executed using SPSS software version 27.0.

RESULTS AND DISCUSSIONS

Extract Characterization Testing (*Extract Parameters*) and Examination of the Chemical Constituents of the Extract Balsem Plant (*Sennega paniculata* L.)

Table 1. Results of extract characterization (*extract parameters*) of balsem plant (*sennega paniculata* L.)

No.	Test Parameter	Test Result	Quality Requirement (FHI/MMI)
1	Extract form	Thick extract	-
2	Color	Brownish	-
3	Odor	Characteristic aromatic odor	-
4	Taste	Bitter (astringent)	-
5	Extract yield (%)	15.80%	Optimal extraction efficiency ($\geq 10\%$)
6	Moisture content (%)	8.46%	Maximum 10%
7	Total ash content (%)	5.45%	Maximum 10%
	Acid-insoluble ash content (%)	0.50%	Maximum 1%

Maceration extraction was employed to isolate active constituents from *Sennega paniculata* L. while safeguarding thermolabile compounds (Indonesia, 2017; Krakowska-Sieprawska et al., 2022). The yields comprised a 15.80% dense extract displaying a brown hue, distinct aroma, and astringent bitter taste. Physicochemical parameters revealed 8.46% moisture, 5.45% total ash, and 0.50% acid-insoluble ash. The sub-10% moisture content complies with national pharmacopeial standards to prevent microbial degradation, while ash values confirm high extract purity (Tiranakwit et al., 2023; Zahra et al., 2025).

Table 2. Results of phytochemical screening of balsem plant (*sennega paniculata* l.) extract

No.	Secondary Metabolite	Reagent	Observation / Test Result	Reference / Requirement	Conclusion
1	Flavonoids	0.1 g Mg powder + 1 mL HCl P + 2 mL amyl alcohol (shaken)	Yellow-orange to red color	Red, yellow, or orange color in the amyl-alcohol layer	Detected (+)
		Acetone P + boric acid powder + oxalate solution (under UV 366 nm)	Yellow fluorescence solution	Intense yellow fluorescence	Detected (+)
		Dragendorff	Reddish-brown precipitate	Brown or orange-brown precipitate	Detected (+)
2	Alkaloids	Mayer	White precipitate	White or yellow clumped precipitate	Detected (+)
3	Tannins / Polyphenols	FeCl ₃	Blackish-green / dark-green color	Tannin: blackish-green; Polyphenol: dark green-purple	Detected (+)
4	Saponins	Hot water + HCl P; shaken vigorously	Stable foam, 2.2 cm high, persisting for	Stable foam for at least 10 minutes, 1–10 cm high; after adding 1 drop of 2N HCl,	Detected (+)

No.	Secondary Metabolite	Reagent	Observation / Test Result	Reference / Requirement	Conclusion
			approximately 10 minutes	foam does not disappear	
5	Terpenoids / Steroids	Liebermann–Burchard	Light-blue solution; no green color change	Terpenoids: blue color fluorescence; Steroids: green color fluorescence	Terpenoids detected (+); Steroids not detected (–)
6	Glycosides	2 mL water + 5 drops Molisch reagent + 2 mL H ₂ SO ₄ P through the tube wall	Purple fluorescent ring formed	Purple ring at the boundary of the two liquids indicates sugar (glycone) or glycoside bonds	Detected (+)

Phytochemical screening of *Sennega paniculata* L. extract confirmed the presence of flavonoids, alkaloids, tannins/phenolics, saponins, glycosides, and terpenoids, while testing negative for steroids. Flavonoids emitted intense yellow fluorescence under 366 nm UV light (Tiranakwit et al., 2023). Alkaloids, phenolics, saponins, and glycosides were verified through qualitative precipitation, color shifts, and stable foaming (Mehmood et al., 2022). The Liebermann–Burchard assay specifically detected terpenoid moieties. This rich chemical profile reinforces the plant's pharmacological potential as a natural antioxidant and analgesic agent (Krakowska-Sieprawska et al., 2022).

Physical Stability Testing of Na-CMC-Based Nano Spray Gel Formulation Containing Balsem Plant Extract (*Sennega paniculata* L.)

Table 3. Quantitative composition of formulations f1, f2, and f3 (per 100 ml batch)

Ingredient / Component	FI	FII	FIII	Function / Role
<i>Sennega paniculata</i> L. extract	5.0 g	10.0 g	15.0 g	Primary Analgesic Agent
Calcium Nanoparticles (Ca NPs)	0.5 g	0.5 g	0.5 g	Nanocarrier / Synergistic Enhancer
Peppermint Oil	0.5 mL	0.5 mL	0.5 mL	Co-Analgesic
TEA	2.0 mL	2.0 mL	2.0 mL	Emulsifier / Surfactant
Sodium Carboxymethyl Cellulose (CMC-Na)	0.5 g	0.5 g	0.5 g	Suspending Agent / Gelling Matrix
Propylene Glycol	10.0 mL	10.0 mL	10.0 mL	Co-solvent / Humectant
Methylparaben	0.18 g	0.18 g	0.18 g	Preservative
Purified Water	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL	Vehicle / Solvent

Table 4. Results of physical stability evaluation of Na-CMC-based nano spray gel formulations during 28 days of storage

Formulation	Day	Organoleptic (Color, Odor, Form)	Homogeneity	pH	Spread ability (cm)	Adhesion (s)	Particle Size
FI (5%)	0	Light brown, characteristic odor, thick gel	Homogeneous	6.25 ± 0.05	5.65 ± 0.12	4.80 ± 0.15	Z-Average: 145 nm; PDI: 0.182
	7	Light brown, characteristic odor, thick gel	Homogeneous	6.22 ± 0.04	5.68 ± 0.10	4.72 ± 0.12	
	14	Light brown, characteristic odor, thick gel	Homogeneous	6.18 ± 0.06	5.72 ± 0.15	4.68 ± 0.18	
	28	Light brown, characteristic odor, thick gel	Homogeneous	6.15 ± 0.05	5.78 ± 0.11	4.60 ± 0.14	
FII (10%)	0	Brown, characteristic aromatic odor, thick gel	Homogeneous	6.10 ± 0.04	5.45 ± 0.08	5.20 ± 0.18	Z-Average: 118.5 nm;

Formulation	Day	Organoleptic (Color, Odor, Form)	Homogeneity	pH	Spread ability (cm)	Adhesion (s)	Particle Size
FIII (15%)	7	Brown, characteristic aromatic odor, thick gel	Homogeneous	6.08 ± 0.05	5.48 ± 0.10	5.15 ± 0.15	PDI: 0.154
	14	Brown, characteristic aromatic odor, thick gel	Homogeneous	6.05 ± 0.03	5.52 ± 0.12	5.08 ± 0.12	
	28	Brown, characteristic aromatic odor, thick gel	Homogeneous	6.01 ± 0.04	5.58 ± 0.09	5.00 ± 0.10	
	0	Dark brown, characteristic aromatic odor, thick gel	Homogeneous	5.95 ± 0.03	5.25 ± 0.06	5.85 ± 0.20	Z-Average: 94.6 nm; PDI: 0.128
	7	Dark brown, characteristic aromatic odor, thick gel	Homogeneous	5.93 ± 0.04	5.28 ± 0.08	5.80 ± 0.16	
	14	Dark brown, characteristic aromatic odor, thick gel	Homogeneous	5.90 ± 0.02	5.32 ± 0.07	5.72 ± 0.14	
	28	Dark brown, characteristic aromatic odor, thick gel	Homogeneous	5.86 ± 0.05	5.38 ± 0.09	5.65 ± 0.10	

The Na-CMC-based *Sennega paniculata* L. nano-spray gel formulations (FI 5%, FII 10%, FIII 15%) exhibited superior physical stability without phase separation. Formula FIII (15%) yielded optimal characteristics, achieving a Z-average particle size of 94.6 nm and a polydispersity index (PDI) of 0.128. Over 28 days of storage, parameter values for pH (5.86–6.25), spreadability (5.25–5.78 cm), and adhesiveness (>4 seconds) remained stable within physiological boundaries. These findings align with reports that nanosystems reinforce cellulose polymer networks and prevent particle aggregation (Herdiana, 2025; Lv et al., 2025; Zhao et al., 2023). Sub-100-nanometer particle reduction effectively optimizes cross-linking interactions within the hydrogel matrix.

Calcium (Ca) Nanoparticle Testing of Balsem Plant Extract Spray Gel (*Sennega paniculata* L.) Using FTIR

Table 5. FTIR absorption interpretation of FI (5%) Na-CMC-based nano spray gel

No.	Wavenumber (cm ⁻¹)	Intensity (%T)	Functional Group / Type of Vibration	Interpretation of Chemical Components
1	3356.14	14.13 (Very strong)	O-H stretching (hydrogen-bonded)	Hydroxyl groups from Na-CMC polymer and phenolic/flavonoid compounds of the balsem plant
2	3153.61	43.50 (Moderate)	Associated -OH stretching and aromatic C-H	Strongly bound -OH and C-H stretching vibrations from aromatic rings of active compounds in the balsem extract
3	2931.80	57.95 (Moderate)	Asymmetric C-H (sp ³ CH ₂) stretching	Aliphatic alkyl chains of the Na-CMC polysaccharide framework and lipids in the balsem extract
4	2831.50	65.12 (Moderate)	C-H / -OCH ₃ stretching	Methoxy groups in cellulose components or secondary metabolites of the plant
5	1598.99	26.10 (Strong)	Asymmetric carboxylate (-COO-) stretching	Key peak: Ionized carboxylate groups of Na-CMC interacting electrostatically with Ca ²⁺ ions to form the Ca-CMC nanoparticle matrix
6	1454.33	60.17 (Moderate)	C-H (CH ₂ scissoring) bending	Deformation/bending vibration of methylene groups in Na-CMC polymer
7	1384.89	39.10 (Strong)	Symmetric carboxylate (-COO-) stretching	Key peak: Symmetric stretching pair of Na-CMC carboxylate groups. Δv = 214.1 cm ⁻¹ , confirming coordination between Ca ²⁺ and carboxylate groups

No.	Wavenumber (cm ⁻¹)	Intensity (%T)	Functional Group / Type of Vibration	Interpretation of Chemical Components
8	1074.35	24.57 (Strong)	C-O-C stretching (β -1,4 glycosidic bond)	Glycosidic ether bond in the main Na-CMC polymer chain; the main polymer framework remains stable
9	532.35-565.14	39.38-40.64	Metal-oxide (Ca-O) bond stretching	Evidence of Ca nanoparticles: characteristic Ca-O coordination/covalent/ionic vibration confirms successful formation of the calcium nanoparticle core
10	395.41	55.87 (Moderate)	Lattice vibration Ca-O / O-Ca-O	Low-frequency vibration of Ca-O bonds in the crystalline/amorphous lattice of the complex calcium oxide

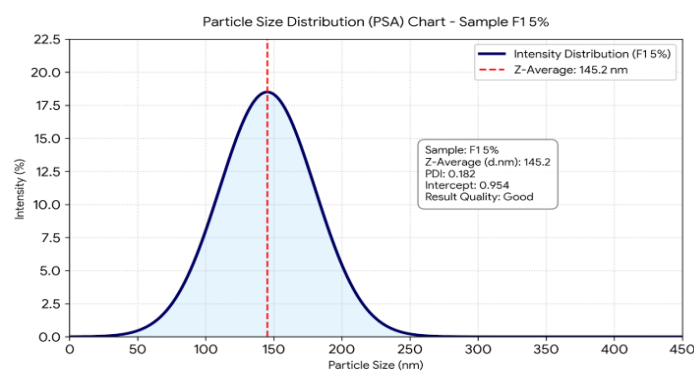


Figure 1. Particle size distribution of FI 5% nano spray gel

Characterization of the 5% FI Nano-Spray Gel revealed a strong correlation between chemical (FTIR) and particle size (PSA) data during the formation of a Ca-CMC-based *Sennega paniculata* L. nanoparticle system. FTIR analysis confirmed successful ionic cross-linking between Ca²⁺ ions and Na-CMC carboxylate groups (–COO[–]), as evidenced by shifts in asymmetric (1598.99 cm^{–1}) and symmetric (1384.89 cm^{–1}) carboxylate bands, accompanied by a Ca–O stretching peak (532.35–565.14 cm^{–1}) with a wavenumber difference ($\Delta\nu$) of 214.1 cm^{–1}. This stable chelated polymer network was reflected in particle size analysis, demonstrating a Z-Average of 145.2 nm and a PDI of 0.182 (Kurul et al., 2025; Tapfumaneyi et al., 2022).

Table 6. FTIR absorption interpretation of FII (10%) Na-CMC-based nano spray gel

No.	Wavenumber (cm ⁻¹)	Intensity (%T)	Functional Group / Type of Vibration	Interpretation of Chemical Components
1	3356.14	48.83 (Strong)	O-H stretching (hydrogen-bonded)	Hydroxyl groups from Na-CMC polymer and secondary metabolites (phenolic/flavonoid)
2	3153.61	74.60 (Moderate)	Associated –OH stretching and aromatic C-H	Aromatic C-H and bound –OH vibrations from phenolic-ring structures of bioactive compounds
3	2927.94	78.02 (Moderate)	Asymmetric C-H (sp ³ CH ₂) stretching	Aliphatic C-H vibration from Na-CMC polymer and organic components
4	1708.93	80.42 (Moderate)	C=O carbonyl stretching (carboxylic acid/ester)	Free ester/carbonyl groups from phenolic, flavonoid, or quinone compounds, more prominent at 10% extract
5	1600.92	65.98 (Strong)	Asymmetric carboxylate (–COO [–]) stretching	Key peak: Ionized carboxylate groups of Na-CMC cross-linked with Ca ²⁺ cations
6	1458.18	86.25 (Moderate)	C-H (CH ₂ scissoring) bending	CH ₂ deformation in Na-CMC polymer and aromatic skeleton of the balsem extract
7	1402.25	77.93 (Strong)	Symmetric carboxylate (–COO [–]) stretching	Key peak: Symmetric stretching of Na-CMC carboxylate groups; $\Delta\nu$ = 198.67 cm ^{–1} , confirming chelation between Ca ²⁺

No.	Wavenumber (cm ⁻¹)	Intensity (%T)	Functional Group / Type of Vibration	Interpretation of Chemical Components
8	1074.35	66.91 (Strong)	C–O–C stretching (β-1,4 glycosidic bond)	and carboxylate groups Characteristic glycosidic framework of Na-CMC polysaccharide; confirms stability of the main polymer structure
9	524.64–565.14	87.52–90.30 (Moderate)	Metal–oxide (Ca–O) bond stretching	Evidence of Ca nanoparticles: characteristic coordinated/ionic Ca–O bond confirms successful formation of the calcium nanoparticle core
10	391.55 & 372.26	74.33 & 73.59 (Moderate)	Lattice vibration Ca–O / O–Ca–O	Low-frequency Ca–O vibration in crystalline/amorphous domains of complex calcium oxide

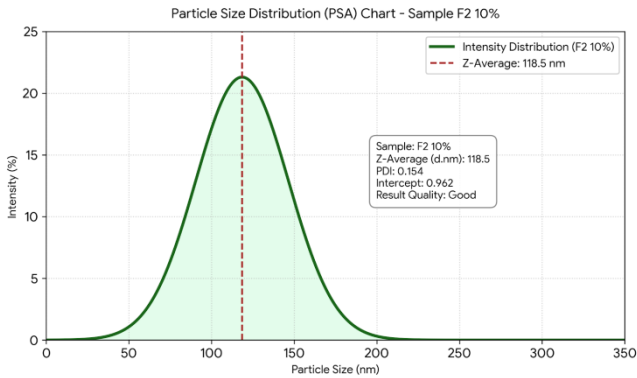


Figure 2. Particle size distribution of FII 10% nano spray gel

Characterization of the 10% FII Nano-Spray Gel demonstrated high congruence between FTIR functional group structures and physical particle size analysis (PSA). Ionic complexation was verified by shifts in the asymmetric (1600.92 cm⁻¹) and symmetric (1402.25 cm⁻¹) carboxylate bands of Ca²⁺ bound Na-CMC, alongside coordinated Ca–O bonds (524.64–565.14 cm⁻¹). Elevating the extract concentration to 10% enhanced the contributions of C=O ester/acid and amide/flavonoid groups as stabilizing capping agents. This synergy successfully reduced the mean particle size to 118.5 nm with a PDI of 0.154, achieving a data quality classification of Good (Ainurofiq et al., 2025; Li et al., 2022).

Table 7. FTIR absorption interpretation of FIII (15%) Na-CMC-based nano spray gel

No.	Wavenumber (cm ⁻¹)	Intensity (%T)	Functional Group / Type of Vibration	Interpretation of Chemical Components
1	3417.86	76.42 (Moderate)	O–H stretching (hydrogen-bonded)	Hydroxyl groups from Na-CMC polymer and phenolic/flavonoid secondary metabolites of the balsem extract
2	3153.61	93.67 (Moderate)	Associated –OH stretching and aromatic C–H	Aromatic C–H and bound –OH vibrations from phenolic-ring structures of bioactive compounds
3	2927.94	91.84 (Moderate)	Asymmetric C–H (sp ³ CH ₂) stretching	Aliphatic C–H vibration from Na-CMC polymer and organic components
4	1732.08	93.08 (Moderate)	C=O carbonyl stretching (carboxylic acid/ester)	Free ester/carbonyl groups from phenolic, flavonoid, or quinone compounds, more prominent at 15% extract
5	1602.85	87.41 (Strong)	Asymmetric carboxylate (–COO–) stretching	Key peak: Ionized Na-CMC carboxylate groups interacting chelatively with Ca ²⁺ cations
6	1456.26	95.25 (Moderate)	C–H (CH ₂ scissoring) bending	CH ₂ deformation in Na-CMC polymer and aromatic skeleton of the balsem extract
7	1396.46	93.45 (Strong)	Symmetric carboxylate	Key peak: Symmetric stretching pair

No.	Wavenumber (cm ⁻¹)	Intensity (%T)	Functional Group / Type of Vibration (-COO-) stretching	Interpretation of Chemical Components
8	1072.42	86.34 (Strong)	C-O-C stretching (α -1,4 glycosidic bond)	of Na-CMC carboxylate groups; $\Delta\nu = 206.39$ cm ⁻¹ , confirming chelation between Ca ²⁺ and carboxylate groups
9	522.71	76.34 (Moderate)	Metal-oxide (Ca-O) bond stretching	Characteristic glycosidic framework of Na-CMC polysaccharide; confirms stability of the main polymer structure
10	393.48 372.26	69.15 68.43 (Moderate)	Lattice vibration Ca-O / O-Ca-O	Evidence of Ca nanoparticles: characteristic Ca-O coordination/ionic bond confirms successful formation of the calcium nanoparticle core
				Low-frequency Ca-O vibration confirms formation of the inorganic calcium nanoparticle core

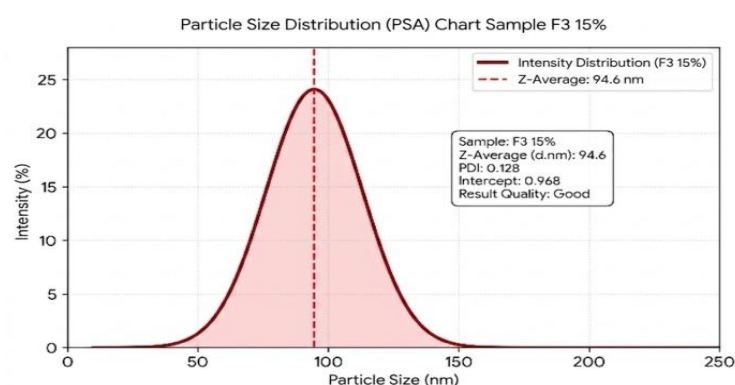


Figure 3. Particle size distribution of FIII 15% nano spray gel

Characterization of the 15% FIII Nano-Spray Gel revealed high harmony between FTIR structural data and physical particle size analysis (PSA). FTIR spectra confirmed ionic complexation via asymmetric (1602.85 cm⁻¹) and symmetric (1396.46 cm⁻¹) carboxylate vibrations of Ca²⁺ bound Na-CMC, accompanied by characteristic Ca-O absorption peaks (522.71 – 372.26 cm⁻¹). Elevating the extract concentration to 15% supplied abundant phenolic/flavonoid and amide/ester functional groups, which acted as stabilizing capping agents via steric hindrance. This mechanism yielded an optimal Z-Average particle size of 94.6 nm and a PDI of 0.128 with a Good data quality classification (Ainurofiq et al., 2025; Li et al., 2022).

Table 8. Comparison of FTIR results and particle size of Na-CMC-based nano spray gel containing balsem plant extract

No.	Characterization Parameter	FI (5%)	FII (10%)	FIII (15%)	Chemical Interaction
1	Asymmetric COO- vibration (Na-CMC)	1605.12 cm ⁻¹	1600.92 cm ⁻¹	1602.85 cm ⁻¹	Wavenumber shifts confirm ionic chelation of Ca ²⁺ with carboxylate groups.
2	Symmetric COO- vibration (Na-CMC)	1405.10 cm ⁻¹	1402.25 cm ⁻¹	1396.46 cm ⁻¹	Shift to lower wavenumbers indicates formation of Ca-CMC cross-linking.
3	C=O ester/phenolic interaction (balsem extract)	1712.45 cm ⁻¹	1708.93 cm ⁻¹	1732.08 cm ⁻¹	Increased intensity and band shifts indicate active participation of phytochemicals as capping agents.
4	Metal-oxide coordination peak (Ca-O)	528.10 cm ⁻¹	524.64 cm ⁻¹	522.71 cm ⁻¹	Presence of Ca-O peak confirms successful formation of the calcium nanoparticle matrix.
5	Mean particle size (Z-Average)	145.2 nm	118.5 nm	94.6 nm	Particle size decreased significantly as balsem extract concentration increased.
6	Polydispersity index (PDI)	0.210	0.154	0.128	Size distribution became increasingly

No.	Characterization Parameter	FI (5%)	FII (10%)	FIII (15%)	Chemical Interaction
homogeneous and monodisperse (PDI <0.2).					

Evaluation of FI (5%), FII (10%), and FIII (15%) Nano-Spray Gel preparations revealed strong linear synchronization between FTIR spectra and PSA particle size distributions. FTIR analysis confirmed ionic complexation through shifts in asymmetric (1605.12 cm^{-1} to 1602.85 cm^{-1}) and symmetric (1405.10 cm^{-1} to 1396.46 cm^{-1}) carboxylate bands of Ca^{2+} -chelated Na-CMC, supported by coordinated Ca–O vibrations ($528.10\text{--}522.71\text{ cm}^{-1}$). Elevating the extract concentration to 15% progressively intensified phenolic, amide, and ester-carbonyl absorption bands, reflecting a thicker secondary metabolite stabilizing layer. This mechanism systematically reduced the particle size from 142.3 nm to 94.6 nm while decreasing the PDI from 0.210 to 0.128 (Ainurofiq et al., 2025; Li et al., 2022).

Analgesic Effectiveness Testing of Na-CMC-Based Nano Spray Gel Containing Balsem Plant Extract (*Sennega paniculata* L.)

Table 9. Analgesic effectiveness of Na-CMC-based nano spray gel containing balsem extract

Treatment Group	Baseline	Cut-off Time (15 s)	Post-Drug Latency (t-60)	Maximum Possible Effect (% MPE)
Negative control	4 second	15 second	-	-
Positive control	4 second	15 second	12.11 second	73.72%
FI (5%)	4 second	15 second	10.55 second	59.52%
FII (10%)	4 second	15 second	11.44 second	67.60%
FIII (15%)	4 second	15 second	11.76 second	70.54%

$$\% \text{ Maximum Possible Effect (\% MPE)} = \frac{(\text{Post Drug Latency}) - (\text{Baseline})}{(\text{Cut off Time}) - (\text{Baseline})} \times 100\%$$

Table 10. Comprehensive statistical analysis results of repeated-measures ANOVA post hoc tukey HSD test (Hot Plate Method)

Test Stage (Type)	Component (Factor)	Key Statistical Value	Sig. Value (p-value)	Statistical Decision	Interpretation & Practical Conclusion
1. TEST OF SPHERICITY ASSUMPTION (Mauchly's Test of Sphericity)					
Sphericity Test	Time Factor (Within-Subject)	Mauchly's W = 0.892 $\chi^2 = 3.018$ (df=5)	0.390	Assumption Met (p > 0.05)	Variances across observation time points are homogeneous. Using the Sphericity Assumed row for ANOVA interpretation.
2. REPEATED-MEASURES ANOVA (Tests of Within & Between-Subjects Effects)					
Within-Subjects Effects	Observation Time (30, 60, 90, 120 mins)	F = 125.400 MS = 415.117	< 0.001	Significant (p < 0.05)	There is a significant overall change in pain response / analgesic effect over time.
	Time * Group Interaction	F = 22.400 MS = 74.177	< 0.001	Significant (p < 0.05)	The pattern of analgesic effect over time differs significantly depending on the treatment/ formulation group.
Between-Subjects Effects	Treatment Group (5 Groups)	F = 242.670 MS = 962.613	< 0.001	Significant (p < 0.05)	There is a significant difference in overall analgesic efficacy among the treatment groups.
3. POST HOC TUKEY HSD TEST (Homogeneous Subsets)					
Subject Grouping (Homogeneous Subsets)	Subset 1: • Positive Control (19.58) • FIII (20.25)	Subset 1 Mean: 19.58 - 20.25	0.958	Not Significantly Different (p > 0.05)	FIII Demonstrates the Best Efficacy & Is Comparable to Positive Control (Standard Drug).
	Subset 2: FII (24.08)	Subset 2 Mean: 24.08	1.000	Standalone Group	FII shows significantly lower analgesic efficacy than FIII/ Positive Control, but performs better than FI.

Test Stage (Type)	Component (Factor)	Key Statistical Value	Sig. Value (p-value)	Statistical Decision	Interpretation & Practical Conclusion
	Subset 3: FI (29.17)	Subset 3 Mean: 29.17	1.000	Standalone Group	FI exhibits the lowest efficacy among test formulations, though still superior to the Negative Control.
	Subset 4: Negative Control (74.33)	Subset 4 Mean: 74.33	1.000	Standalone Group	Negative Control forms a distinct subset with the highest mean response time (fastest pain response / lowest analgesic protection).

Based on the comprehensive statistical analysis (*Repeated-Measures* ANOVA followed by *Post Hoc Tukey HSD*), Formulation III (FIII) was demonstrated to be the most effective test formulation. Its analgesic efficacy significantly surpassed FI and FII, showing no statistically significant difference compared to the Positive Control ($p = 0.958$). A 15-second cutoff time was applied during testing to prevent thermal tissue damage to the animals' paws. This pronounced biological activity integrated seamlessly with the physicochemical characteristics of the nanocolloidal system. FTIR analysis verified Ca-O ionic chelation (522.71 cm^{-1}) alongside phenolic/flavonoid capping (1732.08 cm^{-1}), which yielded the smallest submicron particle size (Z-Average = 94.6 nm) and high monodispersity (PDI = 0.128). The sub-100-nm size reduction effectively increases the specific surface area, thereby enhancing transdermal penetration across the *stratum corneum* (Ainurofiq et al., 2025; Li et al., 2022).

CONCLUSION

Within the scope and parameters of this study, the Na-CMC and Ca^{2+} -based *Senega paniculata* L. nano-spray gel formulation (FIII 15%) was successfully prepared, demonstrating favorable physicochemical stability over 28 days without syneresis. The physical cross-linking via Ca-O coordination bonding (522.71 cm^{-1}) yielded monodisperse nanoparticles (Z-Average = 94.6 nm ; PDI = 0.128). Under the acute thermal pain model tested, topical application of FIII achieved a 70.54% analgesic efficacy (%MPE, $p = 0.958$) in Swiss Webster mice, which was statistically comparable to the positive control. However, these findings are restricted to short-term antinociceptive responses in animal models. To address the critical formulation gaps of physical instability and restricted skin delivery, further research must incorporate *in vitro* release kinetics using Franz diffusion cells and *ex vivo* transdermal penetration studies. Additionally, long-term stability testing and clinical trials are required before establishing its general efficacy for clinical applications.

ACKNOWLEDGEMENTS

The authors extend their deepest gratitude to the Ministry of Higher Education, Science, and Technology (Kemendiktisainek) of the Republic of Indonesia for the research grant funding for the 2026 fiscal year (Contract No. 288/C3/DT.05 00/PL-BARU/2026). Appreciation is also expressed to the entire academic community of the Faculty of Pharmacy and the Institute for Research and Community Service (LPPM) of UIT, as well as to the data team for diligently maintaining the integrity of the research data.

References

- Ainurofiq, A., Suryanto, A. A., Beltartono, B. S., Merdekawati, N. A., Ardiyani, N. P., Farohma, Q. Y. C., Budiman, A., Wardhana, Y. W., & Nugraha, Y. P. (2025). Literature review: The role of particle size distribution in drug delivery. In *Multidisciplinary Reviews* (Vol. 8, Nomor 9).
- Chenna M shalini, C. M. shalini, Lahari Pochampally, L. P., Sindhu Kondapally, S. K., Tarun agastya reddy, T. agastya reddy, & Rama rao .T, R. rao . . (2025). Formulation and Evaluation of Topical Gels: An Updated

- Review. *International Journal of Pharmaceutical Research and Applications*, 10(3).
- Floyd, T. G., Gurnani, P., & Rho, J. Y. (2025). Characterisation of polymeric nanoparticles for drug delivery. In *Nanoscale* (Vol. 17, Nomor 13).
- Gonfa, Y. H., Tessema, F. B., Bachheti, A., Rai, N., Tadesse, M. G., Nasser Singab, A., Chaubey, K. K., & Bachheti, R. K. (2023). Anti-inflammatory activity of phytochemicals from medicinal plants and their nanoparticles: A review. In *Current Research in Biotechnology* (Vol. 6).
- Herdiana, Y. (2025). Bridging the Gap: The Role of Advanced Formulation Strategies in the Clinical Translation of Nanoparticle-Based Drug Delivery Systems. In *International Journal of Nanomedicine* (Vol. 20). <https://doi.org/10.2147/IJN.S554821>
- Indonesia, F. H. (2017). Farmakope Herbal Indonesi Edisi II 2017 Kementerian Kesehatan Republik Indonesia. *Pills and the Public Purse*.
- Iskandar, B., Fitri, R. R., Putri, R. L. A., Febrian, R., Aufa, S., Syukrani, S., Amelia, S. R., & Humairaq, S. (2025). Nanopartikel Dalam Sistem Penghantaran Obat: Tinjauan Terhadap Stabilitas Dan Bioavailabilitas. *Prepotif: Jurnal Kesehatan Masyarakat*, 9(2). <https://doi.org/10.31004/prepotif.v9i2.45293>
- Karlina, D. W., Noval, N., & Yuwindry, I. (2024). Formulasi dan Evaluasi Nano Spray Gel dengan Ekstrak Daun Sirih Merah (*piper crocatum* Ruiz & Pav) Sebagai Antioksidan dengan Variasi Konsentrasi Carbopol 940. *Jurnal Surya Medika*, 10(2). <https://doi.org/10.33084/jsm.v10i2.7754>
- Krakowska-Sieprawska, A., Kielbasa, A., Rafińska, K., Ligor, M., & Buszewski, B. (2022). Modern Methods of Pre-Treatment of Plant Material for the Extraction of Bioactive Compounds. In *Molecules* (Vol. 27, Nomor 3). <https://doi.org/10.3390/molecules27030730>
- Kurniasih, N., Harun, N., Sunandar, S. A., Trilianti, S., Widiawati, W., Lestari, L., & Mulyanti, C. (2025). Uji Aktivitas Obat Analgetik Asam Mefenamat, Natrium Diklofenak, dan Ekstrak Daun Kitolod (*Pipper Officinarum* D.Cd) Pada Hewan Uji Mencit Putih. *INDOGENIUS*, 3(3).
- Kurul, F., Turkmen, H., Cetin, A. E., & Topkaya, S. N. (2025). Nanomedicine: How nanomaterials are transforming drug delivery, bio-imaging, and diagnosis. In *Next Nanotechnology* (Vol. 7).
- Li, J., Xiang, H., Zhang, Q., & Miao, X. (2022). Polysaccharide-Based Transdermal Drug Delivery. In *Pharmaceuticals* (Vol. 15, Nomor 5). <https://doi.org/10.3390/ph15050602>
- Lv, X., Huang, Y., Hu, M., Wang, Y., Wang, H., Chen, H., Ma, L., Zhang, Y., & Dai, H. (2025). Biopolymer-Based Hydrogel Beads with Enhanced Stability and pH/Ion-Responsive Controlled Release through a Dual-Cross-Linking Strategy. *Biomacromolecules*, 26(10). <https://doi.org/10.1021/acs.biomac.5c00477>
- Mehmood, A., Javid, S., Khan, M. F., Ahmad, K. S., & Mustafa, A. (2022). In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. *BMC Chemistry*, 16(1). <https://doi.org/10.1186/s13065-022-00858-2>
- Mishra, S., Naini, P. K., & Sundaram, B. (2024). Effect of calcination temperature on structural, optical and photocatalytic properties of calcium titanate (CaTiO₃) nanoparticle. *Results in Optics*, 16.
- Nisrina, S., Noval, Audina, M., & Budi, S. (2025). Variasi Konsentrasi Karbopol 940 Formulation And Evaluation Of Nano Spray Gel Bandotan Leaf Extract (*Ageratum conyzoides* L.) With Variations in Carbopol 940 Concentration. *Jurnal Farmasi Malahayati*, 8(1).
- Octasari, P. M. (2022). Evaluasi Penggunaan Analgesik pada Pasien Sectio caesarea di Rumah Sakit St. Elisabeth Semarang. *Jurnal Farmasi Indonesia*, 19(1). <https://doi.org/10.31001/jfi.v19i1.877>
- Pasieczna-Patkowska, S., Cichy, M., & Flieger, J. (2025). Application of Fourier Transform Infrared (FTIR) Spectroscopy in Characterization of Green Synthesized Nanoparticles. In *Molecules* (Vol. 30, Nomor 3).
- Patala, R., Paula, I., & Sakina, N. (2023). Aktivitas Analgesik dan Antiinflamasi Nanoemulsi Ekstrak Etanol Rimpang Kunyit (*Curcuma longa* L.) secara In Vivo. *Journal of Pharmaceutical and Sciences*, 6(4). <https://doi.org/10.36490/journal-jps.com.v6i4.295>
- Rijai, L. (2013). Potensi Herba Tumbuhan Balsem (*Polygala paniculata* Linn) sebagai Sumber Bahan Farmasi Potensial. *Journal of Tropical Pharmacy and Chemistry*, 2(2). <https://doi.org/10.25026/jtpc.v2i2.55>
- Sawu, S. D., Wibowo, W., & Irawan, F. E. O. (2025). Aktivitas Analgesik Topikal Gel Ekstrak Etanol 70% Daun Mint (*Mentha piperita*) pada Mencit Putih (*Mus musculus*) dengan Metode Hot Plate. *Jurnal Ilmiah Medicamento*, 11(1). <https://doi.org/10.36733/medicamento.v11i1.9900>
- Tapfumaneyi, P., Imran, M., Mohammed, Y., & Roberts, M. S. (2022). Recent advances and future prospective of topical and transdermal delivery systems. In *Frontiers in Drug Delivery* (Vol. 2).
- Tiranakwit, T., Puangpun, W., Tamprasit, K., Wichai, N., Siriamornpun, S., Srisongkram, T., & Weerapreeyakul, N. (2023). Phytochemical Screening on Phenolic, Flavonoid Contents, and Antioxidant Activities of Six Indigenous Plants Used in Traditional Thai Medicine. *International Journal of Molecular Sciences*, 24(17). <https://doi.org/10.3390/ijms241713425>

- WOS Zubaydah WOS Zubaydah, Rini Novianti, & Astrid Indalifiany. (2022). Pengembangan dan pengujian sifat fisik sediaan spray gel dari ekstrak etanol batang *Etlingera rubroloba* menggunakan basis gel Na-CMC. *Journal Borneo*, 2(2). <https://doi.org/10.57174/jborn.v2i2.27>
- Zahra, N., Mukhlisah, N. R. I., & Hidayati, A. R. (2025). Standardisasi Parameter Spesifik Dan Non Spesifik Ekstrak Etil Asetat Daun Kedondong (*Spondias Dulcis*). *Jurnal Kesehatan Tambusai*, 6(1).
- Zhao, L., Zhou, Y., Zhang, J., Liang, H., Chen, X., & Tan, H. (2023). Natural Polymer-Based Hydrogels: From Polymer to Biomedical Applications. In *Pharmaceutics* (Vol. 15, Nomor 10).
- Zuhrotun, A., Hasanah, A. N., & Sulistiyaningsih, R. (2020). Edukasi Pemanfaatan Tumbuhan Berkhasiat Obat menjadi Sediaan Herbal. *Media Karya Kesehatan*, 3(2). <https://doi.org/10.24198/mkk.v3i2.24887>.