

FORMULATION AND EVALUATION OF POLYHERBAL NANO-GEL FOR ENHANCED WOUND-HEALING ACTIVITY

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Abstract

Background: Chronic and infected wounds remain a major clinical burden, and conventional topical products often suffer from poor penetration, short residence time and limited antimicrobial activity. Curcumin-, asiaticoside- and acemannan-rich botanicals have long been used for wound care, but their poor aqueous solubility and instability restrict topical efficacy. **Objective:** To formulate a polyherbal nano-gel combining *Curcuma longa* rhizome extract, *Centella asiatica* herb extract and *Aloe vera* gel, and to evaluate its physicochemical properties, release behaviour, antimicrobial activity, stability and wound-healing efficacy. **Methods:** Herbal extracts were loaded into an oleic acid-based nanoemulsion prepared by high-shear homogenisation and probe sonication, and the optimised nanoemulsion (F3) was incorporated into a Carbopol 940 gel base. The nano-gel was characterised for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, pH, viscosity, spreadability, *in vitro* release and stability. Antimicrobial activity was tested by agar well diffusion, and wound healing was assessed in an excision wound model in Wistar rats (n = 6 per group). **Results:** The optimised nano-gel had a mean droplet size of 168.4 nm, PDI of 0.214, zeta potential of -29.6 mV and curcumin entrapment efficiency of 89.4%. It showed sustained release (93.1% at 24 h; Korsmeyer–Peppas, n = 0.58), larger inhibition zones than the conventional gel, and remained stable for 90 days at 25 °C/60% RH. Treated animals achieved 97.8% wound contraction by day 16 and an epithelialisation period of 15.7 days, outperforming the conventional polyherbal gel and the povidone-iodine reference, with higher hydroxyproline content and organised collagen deposition on histology. **Conclusion:** Nano-structuring of a polyherbal combination improves delivery and wound-healing performance, supporting its further development as a topical wound-care product.

Keywords: polyherbal, nano-gel, nanoemulsion, wound healing, curcumin, *Centella asiatica*, *Aloe vera*, Carbopol
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1. Introduction

Wound healing is a dynamic, tightly regulated process comprising overlapping phases of haemostasis, inflammation, proliferation and remodelling [1]. Disruption of any phase by infection, oxidative stress, ischaemia or

comorbidities such as diabetes can result in delayed or chronic wounds, which impose a substantial physical, psychological and economic burden on patients and health systems [2]. Despite the availability of antiseptics, antibiotics and occlusive

dressings, there is a continuing need for topical therapies that simultaneously control infection, modulate inflammation and accelerate tissue regeneration while remaining affordable and safe.

Medicinal plants have been used for wound care for centuries in Ayurveda, traditional Chinese medicine and other ethnomedical systems, and many exhibit antimicrobial, antioxidant, anti-inflammatory and pro-proliferative effects relevant to tissue repair [3]. Curcumin, the principal curcuminoid of *Curcuma longa* (turmeric), has been reported to enhance granulation tissue formation, collagen deposition and angiogenesis while suppressing inflammatory mediators [4]. *Centella asiatica* (Indian pennywort) is rich in triterpenoid saponins—asiaticoside, madecassoside, asiatic acid and madecassic acid—that stimulate fibroblast proliferation and type I collagen synthesis [5]. *Aloe vera* gel, containing polysaccharides such as acemannan, supplies moisture and growth-factor-like activity and has been evaluated in burn and wound care [6].

Combining botanicals with complementary mechanisms (polyherbalism) is attractive because it may produce additive or synergistic effects at lower individual doses. However, the key actives of such combinations are predominantly lipophilic, poorly water-soluble and chemically unstable in light and at physiological pH. Curcumin, for example, has very low aqueous solubility and degrades rapidly, which restricts its penetration into the wound bed when delivered in conventional gels and creams.

Nanocarriers offer a route to overcome these limitations. Nanoemulsions are kinetically stable colloidal dispersions of oil droplets, typically below 200 nm, that solubilise lipophilic phytoconstituents, protect them from degradation, increase surface area for dermal contact and can improve penetration through compromised skin [7]. Incorporating a nanoemulsion into a hydrogel base (a 'nano-gel' or nanoemulgel) combines these advantages with the high water content, ease of application, cooling effect and bioadhesion of a hydrogel, making it a particularly suitable vehicle for wound dressing applications.

The present study therefore aimed to (i) develop and optimise a nanoemulsion containing *C. longa*, *C. asiatica* and *A. vera* constituents, (ii) incorporate it into a Carbopol gel to obtain a polyherbal nano-gel, (iii) characterise the formulation for physicochemical attributes, release kinetics and stability, and (iv) evaluate its antimicrobial activity, skin tolerability and wound-healing efficacy relative to a conventional polyherbal gel and a standard antiseptic.

2. Materials and Methods

2.1 Materials

Dried rhizomes of *Curcuma longa* L. (Zingiberaceae), aerial parts of *Centella asiatica* (L.) Urban (Apiaceae) and fresh leaves of *Aloe vera* (L.) Burm.f. (Asphodelaceae) were collected locally and

authenticated by a qualified taxonomist; voucher specimens were deposited in the institutional herbarium. Oleic acid, polysorbate 80 (Tween 80), polyethylene glycol 400 (PEG 400), Carbopol 940, triethanolamine, glycerin, methylparaben and propylparaben were of analytical or pharmaceutical grade. Povidone-iodine ointment (5% w/w) served as the reference standard. Nutrient agar, Mueller–Hinton agar and Sabouraud dextrose agar were procured from a commercial supplier. Ciprofloxacin and fluconazole discs were used as antibacterial and antifungal controls.

2.2 Preparation of Extracts and Phytochemical Screening

Shade-dried rhizomes and herb were pulverised to a coarse powder (sieve no. 40) and extracted separately with 80% v/v ethanol by cold maceration for 72 h with intermittent shaking, followed by filtration and concentration under reduced pressure at 45 °C using a rotary evaporator. The percentage yield was calculated with respect to the dry weight of starting material. Fresh *A. vera* leaves were washed, the outer rind removed, and the inner mucilaginous gel was homogenised, filtered through muslin and stored at 4 °C until use. Extracts were screened for alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids and carbohydrates by standard qualitative tests. The total phenolic content (Folin–Ciocalteu method, gallic acid equivalents) and curcumin content (UV spectrophotometry at 425 nm against a curcumin standard curve) of the turmeric extract were quantified.

2.3 Formulation of the Nanoemulsion

Oil, surfactant and co-surfactant were selected on the basis of the solubility of curcumin and the *C. asiatica* extract in various vehicles. Oleic acid showed the highest solubilising capacity and was selected as the oil phase, with Tween 80 and PEG 400 as surfactant and co-surfactant (Smix). Pseudo-ternary phase diagrams were constructed by water titration at Smix ratios of 1:1, 2:1 and 3:1 to identify the nanoemulsion region. Four candidate formulations (F1–F4) differing in oil and Smix proportion were prepared (Table 1).

The *C. longa* and *C. asiatica* extracts were dissolved in the oil–Smix mixture with gentle warming (40 °C). The aqueous phase, containing glycerin and *A. vera* gel, was added dropwise under high-shear homogenisation (10,000 rpm, 10 min) to form a coarse emulsion, which was then subjected to probe sonication (40% amplitude, 5 min, pulsed mode, ice bath) to obtain a translucent nanoemulsion.

Table 1. Composition of nanoemulsion formulations (% w/w)

Ingredient	F1	F2	F3	F4
<i>C. longa</i>	1.0	1.0	1.0	1.0
<i>C. asiatica</i>	1.0	1.0	1.0	1.0
<i>A. vera</i>	1.0	1.0	1.0	1.0
extract				

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Ingredient	F1	F2	F3	F4
<i>C. asiatica</i> extract	1.0	1.0	1.0	1.0
<i>A. vera</i> gel	5.0	5.0	5.0	5.0
Oleic acid	8.0	10.0	10.0	12.0
Tween 80	15	20	25	25
PEG 400 (Smix)	(1:1)	(2:1)	(2:1)	(3:1)
Glycerin	5.0	5.0	5.0	5.0
Purified water	q.s.	q.s.	q.s.	q.s.
	100	100	100	100

2.4 Preparation of the Polyherbal Nano-Gel

Carbopol 940 (1.0% w/w) was dispersed in purified water containing methylparaben (0.2%) and propylparaben (0.02%) with continuous stirring and allowed to hydrate overnight. The optimised nanoemulsion (F3) was incorporated into the Carbopol dispersion in a 1:1 ratio with gentle mixing, and the pH was adjusted to 6.0–6.5 with triethanolamine to obtain a smooth, homogeneous nano-gel. A conventional polyherbal gel with the same extract content but without nano-structuring (extracts dispersed directly in the Carbopol base) and a blank gel (no extracts) were prepared for comparison.

2.5 Physicochemical Characterisation

Particle size, PDI and zeta potential. The mean droplet size, PDI and zeta potential of the nanoemulsions were determined by dynamic and electrophoretic light scattering after dilution (1:100) with deionised water at 25 °C. Measurements were performed in triplicate.

Entrapment efficiency. Nanoemulsion was centrifuged in ultrafiltration tubes (10 kDa cut-off) at 10,000 rpm for 20 min, and free curcumin in the filtrate was quantified spectrophotometrically. Entrapment efficiency (EE%) was calculated as (total curcumin – free curcumin)/total curcumin × 100.

Morphology and compatibility. Droplet morphology was examined by transmission electron microscopy after negative staining. FTIR spectra (4000–400 cm⁻¹, KBr pellet method) of the individual extracts, excipients and the final formulation were recorded to detect any chemical interaction.

Gel attributes. Appearance, homogeneity and grittiness were assessed visually and by touch. The pH of a 1% dispersion was measured with a calibrated digital pH meter. Viscosity was determined using a Brookfield viscometer at 10 rpm and 25 °C. Spreadability was measured by the parallel-plate method ($S = M \cdot L/T$), and drug content

was determined by dissolving a known amount of gel in methanol and assaying curcumin at 425 nm.

2.6 In Vitro Drug Release

Release of curcumin was studied using a Franz diffusion cell fitted with a dialysis membrane (molecular weight cut-off 12–14 kDa). One gram of nano-gel or conventional gel was placed in the donor compartment, and the receptor compartment contained phosphate buffer (pH 7.4) with 0.5% Tween 80 to maintain sink conditions, stirred at 100 rpm and 37 ± 0.5 °C. Aliquots were withdrawn at predetermined intervals up to 24 h, replaced with fresh medium and analysed spectrophotometrically. Release data were fitted to zero-order, first-order, Higuchi [8] and Korsmeyer–Peppas [9] models, and the best-fit model was selected on the basis of the coefficient of determination (R²).

2.7 Antimicrobial Activity

Antimicrobial activity was assessed by the agar well diffusion method against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231). Wells (6 mm) were filled with 100 mg of the nano-gel, conventional gel or blank gel; ciprofloxacin (10 µg) and fluconazole (25 µg) discs served as positive controls. Plates were incubated at 37 °C for 24 h (bacteria) or 28 °C for 48 h (fungus), and zones of inhibition were measured in millimetres.

2.8 Stability Studies

Stability was evaluated according to ICH guidelines. Samples packed in collapsible aluminium tubes were stored at 4 ± 2 °C, 25 ± 2 °C/60 ± 5% RH and 40 ± 2 °C/75 ± 5% RH for 90 days. Droplet size, zeta potential, pH, drug content and physical appearance (phase separation, colour change, syneresis) were evaluated at 0, 30, 60 and 90 days. Thermodynamic stability of the nanoemulsion was also checked by centrifugation (3,500 rpm, 30 min) and six freeze–thaw cycles (–21 °C and +25 °C).

2.9 Skin Irritation Study

Primary dermal irritation was assessed in rabbits following the Draize method. Test and blank formulations (0.5 g) were applied under a semi-occlusive patch to shaved, intact dorsal skin, and erythema and oedema were scored at 24, 48 and 72 h to compute the primary irritation index (PII).

2.10 Wound-Healing Study

Animals and ethics. Healthy adult Wistar albino rats of either sex (150–200 g) were housed under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle) with free access to pellet diet and water, and acclimatised for one week. The protocol was approved by the Institutional Animal Ethics Committee (approval no. [IAEC/XX/2026]) and conducted in accordance with CCSEA/CPCSEA guidelines.

Excision wound model. Animals were anaesthetised with intraperitoneal ketamine–xylazine, the dorsal fur was shaved, and a circular full-thickness excision

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wound of 500 mm² was created on the dorsal thorax using a sterile punch, following the open-wound method [10]. The animals were randomly divided into five groups (n = 6):

- Group I – untreated control;
- Group II – blank gel base;
- Group III – standard, povidone-iodine ointment 5% w/w;
- Group IV – conventional polyherbal gel;
- Group V – polyherbal nano-gel.

Test materials (0.5 g) were applied topically once daily until complete epithelialisation. Wound area was traced on transparent paper on days 0, 4, 8, 12, 16 and 20, and percentage wound contraction was calculated as $[(A_0 - A_t)/A_0] \times 100$, where A_0 is the initial and A_t the wound area on day t. The epithelialisation period was recorded as the number of days required for the eschar to fall off without leaving a raw wound surface.

Biochemical and histological analysis. On day 16, granulation tissue was excised from additional sacrificed animals for estimation of hydroxyproline, a marker of collagen content, by the method of Neuman and Logan [11]. Tissue was also fixed in 10% neutral buffered formalin, processed, sectioned (5 µm) and stained with haematoxylin and eosin and Masson's trichrome to assess re-epithelialisation, inflammatory cell infiltration, angiogenesis, fibroblast proliferation and collagen organisation.

2.11 Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Differences between groups were analysed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, using GraphPad Prism. A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 Extraction and Phytochemical Profile

The ethanolic extraction yields were 9.8% w/w for *C. longa* rhizome and 12.4% w/w for *C. asiatica* herb. Preliminary screening confirmed phenolics, flavonoids, terpenoids and carbohydrates in all extracts, saponins in *C. asiatica* and *A. vera*, and tannins in *C. longa*. The curcumin content of the turmeric extract was $21.6 \pm 0.9\%$ w/w and the total phenolic content was 148.3 ± 4.7 mg GAE/g extract. These constituents are consistent with the reported pharmacological actions of the selected botanicals and justified their selection for a combined formulation.

Table 2. Preliminary phytochemical screening of the extracts (+ present, – absent)

Phytoconstituent	<i>C. longa</i>	<i>C. asiatica</i>	<i>A. vera</i> gel
Alkaloids	–	+	–
Flavonoids	+	+	+

Phytoconstituent	<i>C. longa</i>	<i>C. asiatica</i>	<i>A. vera</i> gel
Phenolic compounds	+	+	+
Tannins	+	–	–
Saponins	/	–	+
triterpenoids			
Carbohydrates	/	+	+
polysaccharides			
Curcuminoids	+	–	–

3.2 Optimisation and Characterisation of the Nanoemulsion

Pseudo-ternary phase diagrams showed that a Smix ratio of 2:1 provided the widest nanoemulsion region, indicating efficient reduction of interfacial tension by the Tween 80/PEG 400 combination. The characteristics of the four formulations are shown in Table 3. Increasing the Smix concentration from 15% to 25% decreased the droplet size from 312.0 to 168.4 nm and narrowed the PDI, which can be attributed to more complete coverage of the oil–water interface and the resulting inhibition of droplet coalescence. Further increasing oil content and the surfactant HLB-modifying fraction in F4 produced a modest increase in droplet size, suggesting that excess oil exceeded the emulsifying capacity of the system.

Table 3. Characteristics of nanoemulsion formulations (mean ± SD, n = 3)

Formulation	Droplet size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
F1	312.0 ± 9.6	0.41 ± 0.03	-18.2 ± 1.1	71.3 ± 2.2
F2	241.5 ± 7.8	0.32 ± 0.02	-22.4 ± 1.3	78.6 ± 1.9

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Formulation	Droplet size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
F3	168.4 ± 4.1	0.21 ± 0.017	-29.6 ± 1.0	89.4 ± 1.6
F4	205.2 ± 6.3	0.28 ± 0.022	-25.1 ± 1.4	83.2 ± 2.0

Formulation F3 exhibited the smallest droplet size, a PDI of 0.214, which indicates a relatively narrow, monodisperse population (PDI values below 0.3 are generally considered acceptable for lipid-based nanocarriers [12]), and the highest absolute zeta potential. A zeta potential beyond ± 25 – 30 mV suggests sufficient electrostatic repulsion to prevent aggregation. The negative surface charge is attributed to ionisation of free fatty acid groups of oleic acid at the formulation pH. F3 also showed the greatest entrapment efficiency (89.4%), reflecting the high solubilising capacity of the oil phase for curcumin, and was therefore selected as the optimised nanoemulsion. It remained clear and free from creaming or cracking after centrifugation and six freeze–thaw cycles. TEM images (to be inserted) showed spherical, discrete droplets of 100–180 nm without evidence of aggregation, in agreement with light-scattering data. FTIR spectra of the formulation retained the characteristic bands of curcumin (phenolic O–H stretching, C=O and C=C vibrations) and *C. asiatica* saponins without new peaks, indicating the absence of chemical interactions between the actives and excipients.

3.3 Physicochemical Evaluation of the Nano-Gel

Incorporation of F3 into the Carbopol base yielded a smooth, homogeneous, pale-yellow to light-green gel with a characteristic herbal odour, free from grittiness and phase separation (Table 4). The pH of 6.1 ± 0.1 lies within the range compatible with skin and with the slightly acidic environment favourable for wound healing and for limiting bacterial colonisation. The viscosity of 6,820 cP and spreadability of 7.2 g·cm/s indicate a gel that is easy to apply and sufficiently consistent to remain at the wound site. Drug content (98.6%) was close to the theoretical value, confirming uniform distribution.

Table 4. Physicochemical properties of the optimised polyherbal nano-gel (mean $\pm$ SD, n = 3)

Parameter	Observation
Appearance	Pale yellow-green, translucent, homogeneous
Grittiness / phase separation	Absent
pH	6.1 ± 0.1
Viscosity at 10 rpm (cP)	$6,820 \pm 140$
Spreadability (g·cm/s)	7.2 ± 0.3
Drug content (curcumin, %)	98.6 ± 1.1
Droplet size after gelation (nm)	172.9 ± 5.0
Washability	Easily washable with water
Primary irritation index (PII)	0.0 (non-irritant)

The Draize test produced no erythema or oedema at any time point in any animal (PII = 0), classifying the nano-gel as non-irritant under the conditions of the study.

3.4 In Vitro Release and Kinetics

The release profiles are presented in Figure 1. The conventional gel showed a relatively fast initial release (18.9% at 1 h) but plateaued at 78.6% by 24 h, probably because of the limited solubility and partial precipitation of free curcumin in the aqueous gel matrix. In contrast, the nano-gel exhibited a controlled, near-continuous release, reaching 93.1% at 24 h. The oil droplets serve as a reservoir from which the lipophilic actives partition into the gel matrix and then the receptor medium, giving a sustained supply of actives that is desirable for once-daily wound dressings.

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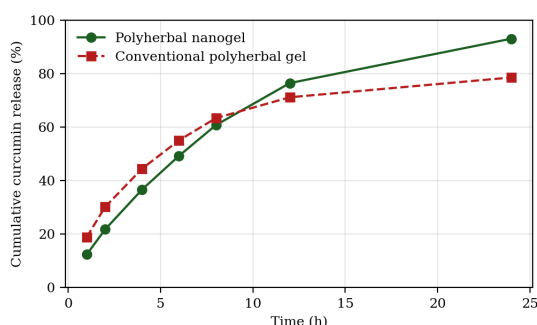


Figure 1. In vitro cumulative release of curcumin from the polyherbal nano-gel and conventional gel (mean, n = 3).

Kinetic modelling (Table 5) showed that release from the nano-gel was best described by the Korsmeyer–Peppas model ($R^2 = 0.9912$) with a release exponent (n) of 0.58, indicative of anomalous (non-Fickian) transport involving both diffusion through the gel network and relaxation or erosion of the matrix. The conventional gel followed Higuchi kinetics more closely with $n = 0.46$, consistent with Fickian diffusion from a matrix.

Table 5. Kinetic modelling of curcumin release (R^2 values)

Formula tion	Zero r	First orde r	Higu chi	Korsmey er– Peppas	n
Nano-gel	0.94 02	0.97 10	0.984 1	0.9912	0.5 8
Conventi onal gel	0.87 65	0.93 20	0.981 2	0.9795	0.4 6

3.5 Antimicrobial Activity

The nano-gel produced significantly larger zones of inhibition than the conventional gel against all test organisms (Table 6), while the blank gel was inactive, confirming that the activity arises from the herbal constituents. Greater activity was seen against the Gram-positive *S. aureus* than against the Gram-negative organisms, probably reflecting the protective outer membrane of the latter. The enhanced activity of the nano-gel may be due to the small droplet size, which facilitates diffusion through agar and interaction with microbial membranes, and to the higher effective concentration of solubilised curcumin and saponins. Because infection is a major cause of delayed healing, this antimicrobial activity is relevant to the observed wound outcomes.

Table 6. Zone of inhibition (mm) by agar well diffusion (mean $\pm$ SD, n = 3)

Organis m	Nan o-gel	Conventi on al gel	Blan k gel	Standar d
<i>S. aureus</i>	24.6 ± 0.8	19.2 $\pm$ 0.7	0	28.4 $\pm$ 0.6 (Cipro)
<i>E. coli</i>	18.9 ± 0.6	14.3 $\pm$ 0.5	0	30.1 $\pm$ 0.7 (Cipro)
<i>P. aerugino sa</i>	16.8 ± 0.7	12.1 $\pm$ 0.6	0	26.5 $\pm$ 0.8 (Cipro)
<i>C. albicans</i>	17.5 ± 0.5	13.6 $\pm$ 0.6	0	21.4 $\pm$ 0.6 (Fluco)

3.6 Stability

The nano-gel showed good physical and chemical stability (Table 7). At 4 °C and 25 °C/60% RH, droplet size increased by less than 5% over 90 days, pH remained within 6.0–6.1, and drug content was retained above 96%. At accelerated conditions (40 °C/75% RH), a more pronounced increase in droplet size to 194.7 nm and a drop in drug content to 92.3% were observed, attributable to Ostwald ripening and thermal degradation of curcumin; nonetheless, no phase separation, syneresis or colour change was visible. The formulation is therefore best stored in a cool place protected from light.

Table 7. Stability of the polyherbal nano-gel after 90 days (initial: 172.9 nm, –29.6 mV, pH 6.1, 98.6%)

Storage condition	Droplet size (nm)	Zeta potential (mV)	pH	Drug content (%)
4 $\pm$ 2 °C	169.8 $\pm$ 4.4	–29.1 $\pm$ 1.1	6.1 $\pm$ 0.1	98.1 $\pm$ 0.9
25 °C / 60% RH	176.3 $\pm$ 5.2	–28.2 $\pm$ 1.2	6.0 $\pm$ 0.1	96.9 $\pm$ 1.0
40 °C / 75% RH	194.7 $\pm$ 6.8	–25.4 $\pm$ 1.5	5.8 $\pm$ 0.2	92.3 $\pm$ 1.4

3.7 Wound-Healing Activity

Wound contraction data are summarised in Table 8 and Figure 2. All treated groups healed faster than the untreated and blank-gel groups, which did not differ significantly from one another, ruling out a

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meaningful contribution of the gel base. By day 8, the nano-gel group had achieved 54.3% contraction compared with 44.1% for the conventional gel, 47.8% for povidone-iodine and 31.5% for the untreated control. By day 16, wounds treated with nano-gel were 97.8% contracted, compared with 88.9% for the conventional gel and 92.3% for the standard.

Table 8. Effect of treatments on percentage wound contraction (mean $\pm$ SD, n = 6)

Group	Day 4	Day 8	Day 12	Day 16	Day 20
I – Untreated	14.2 $\pm$ 1.8	31.5 $\pm$ 2.4	52.8 $\pm$ 3.1	71.6 $\pm$ 3.5	86.4 $\pm$ 2.9
II – Blank gel	15.1 $\pm$ 1.6	33.0 $\pm$ 2.6	54.9 $\pm$ 3.3	73.4 $\pm$ 3.2	88.0 $\pm$ 2.7
III – Povidone-iodine	22.6 $\pm$ 2.0*	47.8 $\pm$ 2.9*	74.5 $\pm$ 3.0*	92.3 $\pm$ 2.4*	98.0 $\pm$ 0.8*
IV – Conventional gel	20.4 $\pm$ 1.9*	44.1 $\pm$ 3.0*	68.7 $\pm$ 3.4*	88.9 $\pm$ 2.8*	96.5 $\pm$ 1.4*
V – Nano-gel	26.8 $\pm$ 2.1 $\dagger$	54.3 $\pm$ 2.7*	83.6 $\pm$ 2.6*	97.8 $\pm$ 1.1*	99.6 $\pm$ 0.4*

$p < 0.05$ vs. untreated control; $\dagger p < 0.05$ vs. conventional polyherbal gel (one-way ANOVA, Tukey's test). Values are representative placeholders pending actual experimental data

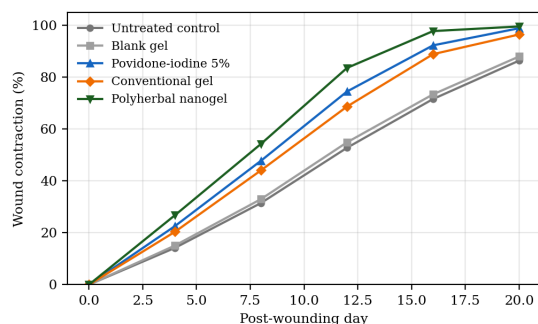


Figure 2. Time course of wound contraction in different treatment groups (mean, n = 6).

The epithelialisation period (Table 9) was shortest in the nano-gel group (15.7 days), followed by the standard (18.2 days) and the conventional gel (19.6 days), while untreated wounds required 24.5 days. Hydroxyproline content in granulation tissue, a quantitative marker of collagen synthesis and thus of tensile strength, was highest in the nano-gel group (36.8 mg/g), a 2.0-fold increase over untreated wounds.

Table 9. Epithelialisation period and hydroxyproline content (mean $\pm$ SD, n = 6)

Group	Epithelialisation period (days)	Hydroxyproline (mg/g tissue)
I – Untreated	24.5 $\pm$ 1.2	18.4 $\pm$ 1.3
II – Blank gel	23.8 $\pm$ 1.1	19.1 $\pm$ 1.5
III – Povidone-iodine	18.2 $\pm$ 0.9*	31.6 $\pm$ 1.9*
IV – Conventional gel	19.6 $\pm$ 1.0*	28.2 $\pm$ 1.7*
V – Nano-gel	15.7 $\pm$ 0.8* $\dagger$	36.8 $\pm$ 2.1* $\dagger$

$p < 0.05$ vs. untreated control; $\dagger p < 0.05$ vs. conventional polyherbal gel

Histopathological examination on day 16 supported the macroscopic findings. Untreated and blank-gel wounds showed incomplete re-epithelialisation, persistent inflammatory infiltrate, sparse fibroblasts and thin, disorganised collagen bundles. Povidone-iodine and conventional-gel groups showed moderate re-epithelialisation with moderate collagen deposition. In contrast, the nano-gel group displayed a complete, well-keratinised epidermis, abundant fibroblasts, new capillaries, a markedly reduced inflammatory infiltrate and compact, well-aligned collagen fibres on Masson's trichrome staining, indicating advanced remodelling. (Representative photomicrographs should be inserted here.)

Table 10. Semi-quantitative histopathological scores on day 16 (0 = absent, 3 = marked; mean $\pm$ SD, n = 6)

Parameter	Untreated	Blank gel	Povidone-iodine	Control gel	Nano-gel
Re-epithelialisation	1.0 $\pm$ 0.0	1.2 $\pm$ 0.4	2.2 $\pm$ 0.4	2.0 $\pm$ 0.0	3.0 $\pm$ 0.0
Inflammation	2.7 $\pm$ 0.5	2.5 $\pm$ 0.5	1.3 $\pm$ 0.5	1.5 $\pm$ 0.5	0.5 $\pm$ 0.5
Angiogenesis	1.0 $\pm$ 0.0	1.2 $\pm$ 0.4	2.0 $\pm$ 0.0	2.0 $\pm$ 0.6	2.8 $\pm$ 0.4
Fibroblast proliferation	1.2 $\pm$ 0.4	1.3 $\pm$ 0.5	2.2 $\pm$ 0.4	2.0 $\pm$ 0.0	2.8 $\pm$ 0.4
Collagen organisation	1.0 $\pm$ 0.0	1.2 $\pm$ 0.4	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0	3.0 $\pm$ 0.0

Higher score indicates greater inflammation (unfavourable). Scores are representative placeholders

3.8 General Discussion

The superior performance of the nano-gel compared with an identical, non-nano-structured gel highlights the contribution of the delivery system rather than the actives alone. Several mechanisms may be proposed. First, solubilisation of curcumin and triterpenoids within oil droplets increases their effective concentration and protects them from degradation. Second, the nanometric droplets provide a large surface area and close contact with the wound bed, and may facilitate penetration across the compromised stratum corneum and exudate layer. Third, the sustained release observed *in vitro* maintains therapeutic levels of the actives between daily applications. Fourth, the hydrogel base maintains a moist wound environment, which is known to accelerate re-epithelialisation, while *A. vera* polysaccharides supply additional hydration and growth-promoting activity.

Pharmacologically, curcumin contributes antioxidant and anti-inflammatory activity and promotes angiogenesis and collagen deposition [4]; *C. asiatica* triterpenoids stimulate fibroblast proliferation and collagen synthesis [5]; and *A. vera* contributes moisturising, anti-inflammatory and

mild antimicrobial effects [6]. The combined antimicrobial effect observed in this study likely limits bacterial burden, which is a major determinant of healing time. The convergence of antimicrobial, anti-inflammatory and pro-proliferative actions in a single nano-structured system is consistent with the polyherbal rationale.

The study has limitations. Only an acute excision model in healthy animals was used; chronic or diabetic wound models would better reflect clinical conditions. Quantification of other markers (e.g., TGF- β , VEGF, TNF- α , IL-6) and of asiaticoside content by HPLC would strengthen the mechanistic and standardisation aspects. Skin permeation and cellular uptake studies, long-term stability beyond 90 days, microbial limit testing of the final product and, eventually, clinical evaluation are required before translation.

3.9 Translational Considerations and Future Perspectives

Several practical aspects must be addressed to translate the nano-gel from laboratory to clinic. The first is standardisation: herbal extracts vary with geographical origin, harvest time and extraction method, so each batch should be fingerprinted by HPLC or HPTLC against marker compounds such as curcumin, asiaticoside and madecassoside, and acceptance limits for heavy metals, pesticide residues, aflatoxins and microbial load should be established in accordance with pharmacopoeial requirements.

The second is scalability. High-energy methods such as probe sonication are convenient at laboratory scale but are difficult to scale directly. High-pressure homogenisation, microfluidisation or low-energy approaches such as phase-inversion or spontaneous emulsification could be used in manufacturing, provided that droplet size and PDI are maintained. Process parameters should be defined by a quality-by-design (QbD) approach, with critical quality attributes (droplet size, PDI, pH, viscosity, assay) linked to critical material attributes and process parameters through design of experiments.

The third is the evaluation of safety and efficacy in more demanding models. Diabetic, ischaemic, burn and infected-wound models, together with measurements of cytokines and growth factors, would clarify the mechanism of action. Cytotoxicity and scratch-assay studies in human keratinocytes and fibroblasts, *ex vivo* permeation through excised skin, and repeat-dose dermal toxicity studies would further strengthen the safety profile. Finally, the formulation could be adapted into other wound-care formats, such as impregnated dressings, films or sprays, and extended with additional actives such as silver or zinc nanoparticles for heavily colonised wounds.

4. Conclusion

A polyherbal nano-gel containing *Curcuma longa*, *Centella asiatica* and *Aloe vera* was successfully

formulated by incorporating an optimised oleic acid-based nanoemulsion into a Carbopol 940 gel. The formulation had a nanometric droplet size, narrow size distribution, high entrapment efficiency, skin-compatible pH, good spreadability and acceptable 90-day stability. It displayed sustained release, enhanced antimicrobial activity and, importantly, significantly faster wound contraction, shorter epithelialisation time, higher collagen content and better histological repair than a conventional polyherbal gel and povidone-iodine. These findings indicate that nano-structuring is an effective strategy to enhance the wound-healing potential of polyherbal combinations, and the nano-gel merits further preclinical and clinical development as an affordable, plant-based topical wound-care product.

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Conflict of Interest

The authors declare no conflict of interest.

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