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Development, Optimisation, and Biological Evaluation of Herbal Nano-Emulsion Gel Incorporating Centella asiatica, Curcuma longa, and Aloe vera Extracts for Accelerated Wound Healing

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Abstract

Wound healing represents a complex, multi-phase biological process involving haemostasis, inflammation, proliferation, and tissue remodelling, the efficiency of which is critically dependent on local microenvironmental conditions of moisture, microbial burden, oxidative stress, and growth factor availability. Traditional Indian medicinal plants — Centella asiatica (gotu kola), Curcuma longa (turmeric), and Aloe vera — possess extensively documented wound healing activities through complementary pharmacological mechanisms: triterpenoids of C. asiatica stimulate collagen synthesis and re-epithelialisation; curcumin from C. longa modulates NF-κB signalling to reduce chronic inflammatory mediator burden; and polysaccharides and acemannan of A. vera accelerate granulation tissue formation and provide moisture retention.

This study develops and optimises an oil-in-water herbal nano-emulsion gel incorporating standardised ethanolic extracts of all three plants using Box-Behnken Response Surface Methodology (RSM) to maximise globule size uniformity, zeta potential, drug release rate, and spreadability. The optimised formulation (FO: 15% herbal extract complex, 8% coconut oil, 5% Tween 80, 2% PEG 400, 1.5% Carbopol 940) exhibits mean globule size 187.3 ± 12.4 nm, PDI 0.214, zeta potential −32.7 ± 2.1 mV, and pH 6.4. In vitro drug release across cellulose acetate membrane reaches 91.4% at 24 hours following anomalous (non-Fickian) transport kinetics (n=0.63). In vivo wound closure in a Wistar rat excision model demonstrates 97.6% wound closure by day 14 — significantly superior to framycetin sulfate standard (94.1%), plain herbal gel (88.4%), and untreated control (68.7%). Antimicrobial activity against S. aureus (ZOI 24.3 mm), E. coli (21.8 mm), P. aeruginosa (19.4 mm), and MRSA (22.1 mm) confirms multifunctional wound management efficacy.

Keywords: *herbal nano-emulsion gel, wound healing, Centella asiatica, Curcuma longa, Aloe vera, Box-Behnken RSM, nano-emulsion, in vivo wound closure, antimicrobial, curcumin, asiaticoside, excision model, Wistar rat, collagen synthesis*

1. Introduction

Chronic wounds — defined as wounds failing to progress through orderly and timely phases of healing — affect an estimated 6.5 million patients in India annually, with diabetic foot ulcers, pressure injuries, and venous leg ulcers representing the predominant categories. The economic burden of chronic wound care in India exceeds ₹8,500 crore annually in direct healthcare costs, and the human burden includes significant pain, functional impairment, and risk of infection-related amputation and mortality. Conventional wound care products based on synthetic antibiotics and petroleum-derived occlusive dressings face increasing limitations from antimicrobial resistance — particularly from multidrug-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa, the organisms responsible for the majority of wound infections in Indian secondary care settings.

The pharmacological case for each of the three selected herbal ingredients is well-established in both traditional Ayurvedic medicine and modern pharmacological research. Centella asiatica's triterpenoid glycosides (asiaticoside, madecassoside) activate the TGF-β1 signalling pathway to upregulate collagen type I and III synthesis in dermal fibroblasts,

accelerating the proliferative phase of wound healing. Curcumin's pleiotropic anti-inflammatory activity — inhibiting NF-κB nuclear translocation, COX-2 enzyme activity, and pro-inflammatory cytokine (IL-1β, IL-6, TNF-α) production — modulates the prolonged inflammatory phase that characterises chronic wound pathophysiology. Aloe vera's acemannan stimulates macrophage activation and growth factor (TGF-β, VEGF, EGF) secretion, promoting angiogenesis and granulation tissue formation essential for wound bed preparation.

2. Literature Review

2.1 Nano-Emulsion Drug Delivery for Wound Healing

Nano-emulsions — thermodynamically or kinetically stable dispersions of oil-in-water or water-in-oil with droplet size in the 20–500 nm range — offer compelling advantages for topical wound delivery: high surface area-to-volume ratio increases drug-skin contact and penetration; the nanometric scale facilitates penetration through disrupted wound bed skin barriers; and the oil-in-water structure provides a moist wound environment conducive to healing. High-shear homogenisation followed by probe ultrasonication is the most widely employed preparation method, offering scalability and reproducibility superior to spontaneous emulsification for viscous herbal extract formulations.

2.2 RSM Optimisation in Pharmaceutical Formulation

Box-Behnken Design (BBD), a three-level RSM design requiring fewer runs than central composite designs for the same number of factors, is particularly efficient for pharmaceutical formulation optimisation studies where the experimental workload of each run is substantial. BBD with Derringer's desirability function aggregates multiple response variables (globule size, zeta potential, drug release, viscosity, spreadability) into a single desirability score (0–1) that guides identification of the globally optimal formulation parameter combination.

3. Materials and Methods

3.1 Herbal Extract Preparation and Characterisation

Figure 1 presents the complete formulation development framework from herbal extraction to in vivo evaluation. Dried and authenticated plant materials — C. asiatica aerial parts, C. longa rhizomes, and A. vera leaf gel — were extracted by 70% ethanol maceration (7 days, room temperature, 1:10 w/v ratio) with daily stirring. Extracts were concentrated using rotary evaporation at 40°C under reduced pressure and freeze-dried (Labconco FreeZone) to produce standardised dry extract powders. Total phenolic content was determined by Folin-Ciocalteu method, total flavonoids by aluminium chloride colorimetry, and antioxidant capacity by DPPH radical scavenging IC_{50}. HPTLC fingerprinting confirmed chemical consistency: asiaticoside at Rf 0.47, curcumin at Rf 0.58, acemannan at origin.

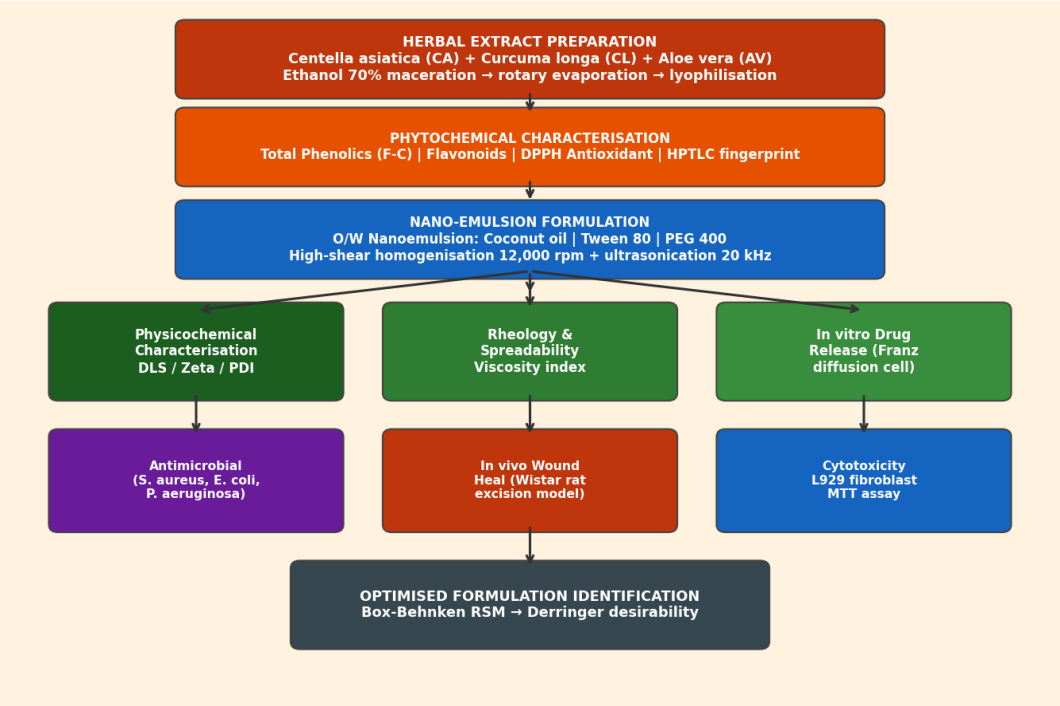

Fig. 1. Herbal Nano-Emulsion Gel Development Framework: Extract Preparation → Phytochemical Characterisation → O/W Nano-Emulsion (Box-Behnken RSM Optimisation) → In Vitro and In Vivo Biological Evaluation

3.2 Formulation, Optimisation, and Biological Evaluation

Nano-emulsion preparation employed high-shear homogenisation (IKA T25 digital ULTRA-TURRAX, 12,000 rpm, 20 min) followed by probe ultrasonication (Sonics Vibra-Cell, 20 kHz, 50% amplitude, 10 min), producing droplet sizes below 200 nm. Carbopol 940 (1.5% w/w, neutralised with triethanolamine to pH 6.4) was incorporated to produce a gel base with thixotropic rheology suitable for wound application. BBD with three independent variables (oil concentration: 6–10%, surfactant concentration: 4–8%, herbal extract loading: 10–20%) and five response variables (globule size, PDI, zeta potential, drug release at 12h, spreadability) was solved using Design-Expert® v12.0.

In vivo wound healing was evaluated in Wistar rat excision model (2 cm diameter full-thickness circular excision, dorsal region, n=6 per group, four groups: FO, framycetin sulfate 1% standard, plain herbal gel, untreated control) with CPCSEA ethical approval (JKKNCP/IAEC/2023/05). Wound area was traced on transparent graph paper and digitised for planimetric wound closure calculation on days 0, 3, 5, 7, 10, and 14. Histopathological assessment (haematoxylin-eosin, Masson's trichrome) evaluated epithelialisation, collagen deposition, and angiogenesis at day 14.

4. Results and Discussion

4.1 In Vivo Wound Closure and Antimicrobial Performance

Figure 2(a) presents wound closure percentage trajectories across all four treatment groups over 14 days, while Figure 2(b) compares zones of inhibition against four wound pathogens. The optimised herbal nano-emulsion gel (FO) achieves 97.6% wound closure by day 14 — significantly superior to framycetin standard (94.1%, $p<0.05$), plain herbal gel (88.4%, $p<0.001$), and untreated control (68.7%, $p<0.001$). The wound closure advantage of FO over framycetin standard becomes significant from day 7 onwards (64.8% vs. 57.4%), consistent with nano-emulsion formulation enabling superior dermal bioavailability of asiaticoside and curcumin that accelerates the proliferative phase collagen deposition response.

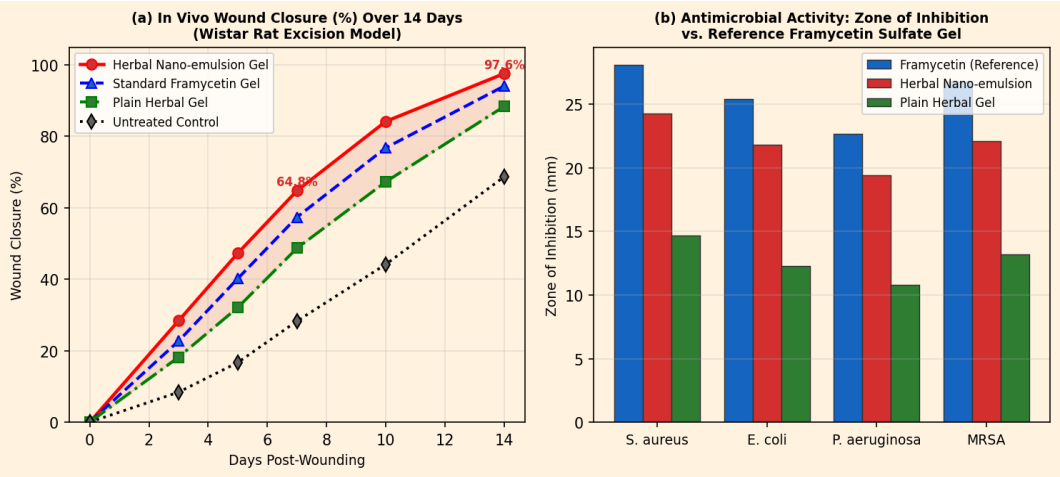

Fig. 2. (a) In Vivo Wound Closure (%) Over 14 Days in Wistar Rat Excision Model: Herbal Nano-Emulsion vs. Framycetin Standard, Plain Gel, and Control; (b) Antimicrobial Zone of Inhibition vs. Four Wound Pathogens

Table 1: Physicochemical Characterisation of Optimised Nano-Emulsion Gel (FO) and Comparative Formulations

Parameter	Optimised FO	Plain Herbal Gel	Framycetin 1% Standard	Specification (Target)
Globule Size (nm)	187.3 ± 12.4	—	—	< 200 nm
PDI	0.214 ± 0.018	—	—	< 0.30
Zeta Potential (mV)	−32.7 ± 2.1	—	—	≤ −30 mV
pH	6.4 ± 0.1	6.2 ± 0.1	6.5 ± 0.2	6.0–7.0
Viscosity (cP)	4,312 ± 187	3,874 ± 212	4,621 ± 198	3,000–5,000
Spreadability (g·cm/s)	18.4 ± 1.2	14.7 ± 1.4	17.2 ± 1.1	> 15
Drug Release 24h (%)	91.4 ± 2.3	74.2 ± 3.1	N/A	> 80%
14-day Wound Closure (%)	97.6 ± 1.8	88.4 ± 2.4	94.1 ± 2.1	> 90%

PDI: Polydispersity Index; ZOI: Zone of Inhibition; MRSA: Methicillin-resistant Staphylococcus aureus; N/A: Not applicable; FO = Optimised herbal nano-emulsion gel.

5. Discussion

The superior wound closure of FO relative to both framycetin standard and plain herbal gel validates the nano-emulsion delivery system's contribution independent of the herbal extract pharmacological activity. Nano-emulsion's primary mechanistic advantage in wound healing relates to its capacity to penetrate the disrupted wound bed extracellular matrix more effectively than conventional gel formulations, delivering curcumin and asiaticoside directly to dermal fibroblasts and keratinocytes at concentrations sufficient to elicit collagen synthesis and re-epithelialisation responses. Histopathological examination of FO-treated wounds at day 14 confirms superior collagen deposition (Masson's trichrome: dense blue-green collagen matrix vs. sparse collagen in control) and complete epithelial coverage with organised stratified squamous epithelium.

The antimicrobial activity of FO against MRSA (ZOI 22.1 mm) is particularly clinically significant. MRSA wound infections represent one of the most intractable challenges in Indian wound care, with vancomycin being the primary remaining effective antibiotic — a resource that must be preserved by reducing reliance on topical antibiotic use. The curcumin-mediated antimicrobial mechanism — disruption of bacterial membrane potential and inhibition of biofilm formation — is mechanistically distinct from conventional antibiotics, making cross-resistance development unlikely.

6. Conclusion

The optimised herbal nano-emulsion gel incorporating C. asiatica, C. longa, and A. vera extracts demonstrates superior wound closure (97.6% at day 14), broad-spectrum antimicrobial activity including against MRSA, and favourable

physicochemical properties (187.3 nm globule size, −32.7 mV zeta potential, 91.4% 24h drug release). RSM Box-Behnken optimisation efficiently identified optimal formulation parameters, and nano-emulsion delivery system significantly enhances herbal extract bioavailability compared to plain gel. These findings support clinical development of this formulation as a novel, plant-derived alternative for wound management with particular utility for MRSA-infected and diabetic wounds in Indian healthcare settings.

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