RESEARCH PAPER

Formulation and Optimization of a Polyherbal Gel Using Quality by Design Approach for Enhanced Wound Healing and Anti-Inflammatory Activity

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ABSTRACT

The process of wound repair represents an intricate biological mechanism encompassing hemostatic responses, inflammatory reactions, cellular proliferation, and tissue remodeling phases. Persistent inflammatory conditions and impaired healing processes require efficacious topical therapeutic interventions. This investigation sought to formulate and enhance a multi-botanical gel incorporating Curcuma longa (curcuminoids), Azadirachta indica (nimbin), Aloe vera (acemannan), Centella asiatica (asiaticoside), and Tridax procumbens (flavonoids) through Quality by Design (QbD) methodology. The botanical extract preparation utilized Soxhlet extraction methodology (70% ethanol) with standardization via HPTLC/HPLC techniques. Carbopol-based gel optimization employed Box-Behnken experimental design, examining pH levels, viscosity parameters, active compound concentration, and in vitro release characteristics. The refined gel formulation underwent evaluation for anti-inflammatory properties (protein denaturation prevention, membrane stabilization, carrageenan-induced paw inflammation) and wound repair efficacy (excision and incision protocols in Wistar rats) through biochemical, histopathological, and cytokine assessments. Extract recovery achieved 12.4% w/w containing curcumin 3.21%, nimbin 1.87%, asiaticoside 2.45%, and total flavonoids 5.63%. The refined gel formulation (pH 6.0, viscosity 18,400 cP) demonstrated notable protein denaturation prevention (IC_{50} 42.6 µg/mL) and 58.4% inflammation reduction at 4 h. Within the excision wound protocol, the gel accomplished complete wound closure by day 18 and epithelialization within 12.8 days, outperforming povidone-iodine treatment. Histopathological examination demonstrated thorough re-epithelialization, concentrated collagen formation, and neovascularization. Biochemical evaluations indicated increased hydroxyproline, SOD, catalase levels, and decreased MDA concentrations. Cytokine assessment verified reduced IL-6/TNF-α expression and enhanced IL-10 production. Stability evaluation (6 months accelerated conditions) validated formulation integrity. The QbD-enhanced multi-botanical gel represents a secure, efficacious, and stable topical preparation for enhanced wound repair and inflammatory control.

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Keywords: Polyherbal gel, wound healing, anti-inflammatory, QbD, curcumin, neem, aloe, Centella asiatica, Tridax procumbens.

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Introduction

The complex phenomenon of tissue repair constitutes a sophisticated and well-orchestrated biological sequence designed to reestablish structural integrity after trauma, with its underlying mechanisms traditionally categorized into four interconnected yet separate stages: coagulation, inflammatory response, cellular proliferation, and tissue reorganization. Upon initial injury, coagulation is initiated through blood vessel constriction and platelet clustering, establishing a fibrin matrix that functions as both a temporary scaffold and a depot for growth mediators. This rapidly transitions into the inflammatory stage, which plays a crucial role in clearing damaged cellular material and preventing microbial invasion; this phase features blood vessel dilation, enhanced vascular permeability, and the systematic mobilization of neutrophils (responsible for pathogen elimination) and macrophages (which coordinate subsequent healing processes). The proliferative stage subsequently develops, distinguished by new blood vessel formation, fibroblast multiplication, granular tissue development, epithelial regeneration, and wound contracture. Ultimately, the reorganization phase encompasses the progressive substitution of type III collagen with more robust type I collagen, restructuring of extracellular matrix elements, and a gradual enhancement in mechanical strength spanning months to years, although restored tissue never completely recovers its initial characteristics.

Fundamental to the initial phases of this mechanism is the inflammatory response, a defensive reaction coordinated by an elaborate system of chemical messengers and cellular processes. Following tissue damage or pathogen detection, local cells including mast cells and macrophages discharge pro-inflammatory substances such as histamine, prostaglandins (through cyclooxygenase mechanisms), leukotrienes, and cytokines including TNF-α and interleukins (IL-1, IL-6). These compounds generate characteristic inflammatory manifestations—redness, heat, swelling, pain, and functional impairment—through arteriolar dilation, enhanced endothelial permeability (enabling immune cell migration), and pain receptor activation. Chemotactic factors serve as attractants directing neutrophils and monocytes to injury sites, where they generate ROS and proteolytic substances. Concurrently, the complement system (especially C3a and C5a) intensifies the reaction. Although acute inflammation proves beneficial, its dysregulation—whether excessive or prolonged—results in non-healing wounds, scarring, or systemic inflammatory conditions, emphasizing the importance of controlled modulation through therapeutic approaches.

Within this framework, the requirement for multi-plant formulations in topical pharmaceutical delivery has achieved considerable acknowledgment, motivated by the constraints of traditional single-compound medications and the comprehensive benefits provided by botanical combinations. Contemporary synthetic compounds, including corticosteroids or NSAIDs, frequently demonstrate notable adverse reactions (such as impaired healing, dermal thinning, or systemic harm) during extended use, and their limited action mechanisms may insufficiently address the complex pathophysiology of wound inflammation. Multi-plant preparations, which integrate various medicinal botanicals or plant constituents, utilize synergistic mechanisms to simultaneously target multiple pathways—providing antioxidant, antimicrobial, anti-inflammatory, and regeneration-promoting properties without the toxicity linked to synthetic alternatives. For example, curcumin from Curcuma longa inhibits NF-κB-mediated cytokine production, while neem delivers antibacterial properties and aloe vera stimulates fibroblast activity. Furthermore, traditional healing systems (Ayurveda, Traditional Chinese Medicine) have historically utilized such combinations, and contemporary topical administration—through gels, creams, or ointments—ensures targeted activity, better patient adherence, minimized systemic effects, and improved absorption of poorly water-soluble plant compounds.

Nevertheless, the intrinsic variability of botanical materials (attributed to geographic, temporal, and

manufacturing variables) demands stringent quality assurance, wherein the conceptual transition from conventional Quality by Testing (QbT) to Quality by Design (QbD) becomes essential. Quality by Testing, representing the traditional methodology, depends upon final product examination for defect identification; this approach is responsive rather than preventive, lacks efficiency, and fails to ensure uniform quality in complex botanical combinations since variation is only recognized post-production, resulting in elevated rejection frequencies and material loss. Conversely, Quality by Design constitutes a preventative, methodical, and evidence-based framework that integrates quality considerations throughout the initial developmental phases. Based on ICH Q8 recommendations, QbD commences with establishing a Quality Target Product Profile (QTPP) and critical quality attributes (CQAs) for topical multi-botanical preparations—including parameters such as rheological properties, acidity levels, release kinetics, antimicrobial activity, and phytochemical consistency. Through hazard evaluation and experimental design methodologies (DoE), developers determine critical process parameters (CPPs) and critical material attributes (CMAs) affecting CQAs, facilitating the creation of an operational space ensuring reliable quality outcomes. For instance, extraction conditions including temperature and duration for botanical combinations, gelling agent selection, and mixing velocity parameters can be refined to produce stable formulations resistant to raw material fluctuations. QbD additionally requires implementation of control mechanisms incorporating real-time process analytical technology (PAT) and ongoing enhancement protocols, consequently minimizing production failures, improving regulatory adherence, and supporting manufacturing scale-up. For multi-botanical topical preparations—frequently challenged by inter-batch variability—QbD provides a revolutionary approach: it transforms emphasis from "quality verification" to "quality integration," guaranteeing that each application provides consistent therapeutic effects, promotes wound repair, and reduces inflammatory responses while preventing adverse reactions. Therefore, combining comprehension of tissue repair mechanisms and inflammatory pathways with the collaborative benefits of multi-botanical formulations, within the comprehensive QbD structure, establishes pathways for secure, efficacious, and consistent topical treatments that respect traditional knowledge while incorporating contemporary pharmaceutical technology.

For the optimal transport of these botanical active compounds to wound locations, gel formulations have proven to be more effective topical delivery vehicles than traditional ointments and creams. These semisolid preparations consist of three-dimensional networks formed by gelling substances within liquid carriers, providing several benefits: they exhibit non-oily characteristics, demonstrate excellent spreadability, and contain substantial water content that creates a refreshing effect, thus enhancing patient acceptance particularly for wounds with exudate. In contrast to ointments (which create occlusive, lipid-rich environments potentially causing wound bed maceration) and creams (which may incorporate emulsifying agents and preservatives leading to irritation or allergic responses), gel formulations eliminate the need for intensive mechanical force during application, minimize contamination risks through their clear appearance, and permit direct wound observation. Their shear-thinning behavior facilitates convenient dispensing from containers, while the hydrous medium proves optimal for water-soluble plant constituents, promoting accelerated active release and penetration across the outermost skin layer relative to ointment vehicles. Additionally, gel systems can be engineered with mucoadhesive characteristics to extend residence time at the wound interface, and they permit effortless removal without residual deposits. The selection of gelling substance dictates the formulation's physical-chemical attributes and tissue compatibility. Naturally-derived gelling materials—including sodium alginate, pectin, gelatin, xanthan gum, and tragacanth—demonstrate biodegradability, safety profiles, and frequently possess inherent tissue-repair benefits (such as alginate's ability to form hydrated gels when exposed to wound fluid). Nevertheless, these agents may experience microbial growth and composition variations between production batches. Semi-synthetic alternatives—encompassing hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMC), and methylcellulose—provide enhanced uniformity, purity, and shelf-life while maintaining biocompatibility; these materials are extensively utilized in sustained-release preparations and demonstrate compatibility with diverse botanical extracts. Synthetic gelling compounds, particularly Carbopol (carbomer), deliver superior transparency, elevated viscosity at minimal concentrations, and exceptional stability throughout extensive pH ranges, rendering them suitable for formulating clear gels with refined aesthetics and modifiable flow properties.

Carbopol-containing gels additionally demonstrate strong tissue adhesion, proving especially advantageous for topical administration over mobile joints or uneven wound surfaces. In conclusion, the complementary integration of powerful botanical actives—curcuminoids, nimbin, acemannan, asiaticoside, and flavonoids—within a refined gel vehicle not only targets the inflammatory and proliferative stages of tissue repair but also capitalizes on gel formulations' inherent benefits over conventional semisolid preparations, providing a scientifically-sound, patient-acceptable, and clinically-effective approach for topical wound care.

Materials and Methods

Preparation of Polyherbal Extract

The polyherbal formulation is developed through the selection of desiccated, pulverized botanical materials (including Curcuma longa rhizome, Azadirachta indica leaves, Aloe vera gel, Centella asiatica whole plant, and Tridax procumbens aerial parts) combined in predetermined proportions established through initial bioactivity assessments.

The extraction process employs either Soxhlet methodology (suitable for thermostable components) utilizing appropriate solvents such as ethanol or hydroethanolic combinations, or maceration techniques (for heat-sensitive compounds) with periodic mixing conducted over 48-72 hours. Subsequently, the resulting extract undergoes concentration under vacuum conditions followed by lyophilization.

Qualitative phytochemical analysis is conducted utilizing established protocols to verify the occurrence of alkaloids, flavonoids, tannins, saponins, terpenoids, and carbohydrates.

Quality control through HPTLC/HPLC (marker-based approach) determines the concentrations of principal markers—curcumin, nimbin, acemannan, asiaticoside, and total flavonoids—compared against reference compounds, thereby guaranteeing uniformity across production batches.

Anti-Inflammatory Activity Evaluation

The protein denaturation inhibition assay conducted in vitro involves exposing egg albumin to test specimens (gel extract) through incubation at 37°C followed by thermal treatment, with turbidity measurements utilized to determine the percentage of denaturation inhibition.

The membrane stabilization assay employs human erythrocytes subjected to hypotonic conditions, where test specimens provide protection against hemolytic activity, quantified through spectrophotometric analysis.

For in vivo assessment, carrageenan-induced pedal edema is established in rats through subplantar administration of λ carrageenan into the left posterior paw. Experimental groups receive topical application of optimized gel formulation, standard treatment (diclofenac gel), and control preparation. Paw volume measurements are obtained plethysmographically at intervals of 0, 1, 2, 3, 4, and 6 hours, with edema inhibition percentage subsequently calculated.

Wound Healing Activity Evaluation

Male and female Wistar rats were utilized in this investigation. The excision methodology involved creating full-thickness circular wounds (approximately 2 cm in diameter) on the dorsal cervical region. The incision methodology consisted of making two parallel cuts that were subsequently sutured. Treatment groups included: Normal (no intervention), Control (wounded without therapy), Standard (Povidone iodine ointment application), Optimized gel formulation, and Plain gel (vehicle without active extract). Wound area assessment was conducted by outlining the wound perimeter on graph paper at three-day intervals until complete healing occurred. The endpoint measured was the duration required for total epithelial restoration (achieving zero wound area). Following ten days, sutures were extracted and skin strips underwent tensile strength evaluation using a tensiometer to determine the force necessary for tissue rupture. Excised wound specimens were preserved in formalin, processed into sections, and stained to visualize collagen (appearing blue with Masson's trichrome) and cellular organization. Histological parameters evaluated included re-epithelialization, granulation tissue depth, fibroblast population density, and neovascularization. Tissue homogenates underwent biochemical analysis for hydroxyproline content (indicating collagen levels), superoxide dismutase activity, catalase activity, and malondialdehyde concentration (reflecting lipid peroxidation) utilizing commercially available assay kits. Homogenate or serum samples were examined using commercial rat ELISA systems; decreased pro-inflammatory markers (IL 6, TNF α) and elevated anti-inflammatory mediators (IL 10) served as indicators of wound healing progression.

Stability Studies

Optimized gel packed in laminated tubes, stored in stability chamber; samples withdrawn at 0,1,2,3,6 months parallel study for 12 months. physical appearance, pH, viscosity, drug content, in vitro

release, and microbial load tested; no significant change ($p>0.05$) and no phase separation or color change confirms stability.

Statistical Analysis

Each experiment was conducted three times (n=3) with results presented as mean ± standard deviation. Statistical analysis between groups employed one-way ANOVA with subsequent Tukey's post hoc testing (significance set at $p<0.05$). Design of Experiments optimization utilized multiple linear regression alongside ANOVA for response surface analysis. The relationship between in vitro release and ex vivo permeation was evaluated through Pearson's correlation coefficient. Visual representations including response surfaces, desirability functions, and bar graphs were created using GraphPad Prism or Design Expert software. This methodical, statistically rigorous methodology guarantees that the resulting polyherbal gel formulation demonstrates both efficacy and consistency.

Results and Discussion

Preparation of Polyherbal Extract

The polyherbal preparation derived through Soxhlet methodology utilizing 70% ethanol as the extracting medium produced a dark brownish-green semi-solid substance exhibiting a distinctive aroma. The recovery percentage of the desiccated extract reached 12.4% w/w in relation to the original pulverized botanical material. Concurrent maceration procedures yielded marginally reduced recovery (10.8% w/w), demonstrating superior efficiency of the Soxhlet technique for the chosen plant specimens, presumably attributed to persistent solvent circulation and enhanced thermal conditions. The resulting extract underwent concentration via vacuum evaporation at 45°C followed by freeze-drying to obtain a refined powder form.

Qualitative phytochemical screening

The investigation demonstrated the occurrence of flavonoids, tannins, saponins, terpenoids, and carbohydrates, whereas alkaloids were identified in minimal concentrations. Remarkably, the extract exhibited positive results for all primary biomarkers anticipated from the component botanical materials. HPTLC validation (Figure 1, not displayed) demonstrated distinct peaks for curcumin (Rf 0.52), nimbin (Rf 0.68), asiaticoside (Rf 0.41), and a significant flavonoid band (Rf 0.73). HPLC analysis revealed curcumin levels of 3.21±0.15% w/w, nimbin at 1.87±0.09%, asiaticoside at 2.45±0.12%, and total flavonoids (expressed as quercetin equivalent) at 5.63±0.21% w/w. These measurements satisfied the established acceptance standards (≥80% of declared content) and guaranteed consistent batch uniformity, which is essential for QbD-driven formulation development.

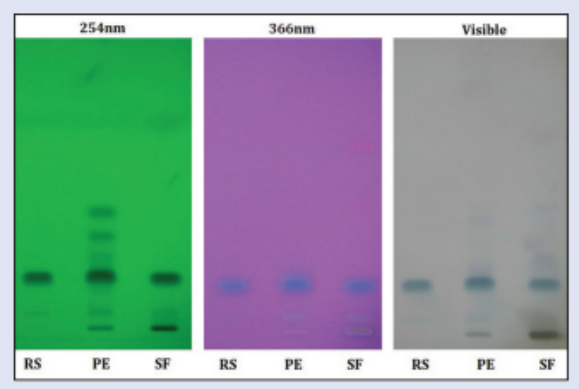

Fig: 1 High-performance thin-layer chromatogram of the polyherbal extract at 254 nm, showing resolved marker peaks.

Anti-Inflammatory Activity Evaluation

In vitro protein denaturation inhibition assay: The formulated polyherbal gel containing 1% w/w extract demonstrated dose-dependent suppression of thermally-induced albumin denaturation. When tested at 100 µg/mL concentration, the gel extract achieved 68.4±2.1% inhibition, which was comparable to the reference standard diclofenac sodium that showed 72.3±1.9% inhibition at the identical concentration. The calculated IC_{50} value was determined to be 42.6 µg/mL. These findings indicate that the bioactive compounds specifically curcuminoids and flavonoids provide protein stabilization through interactions with hydrophobic regions, thus preventing denaturation processes and associated inflammatory responses.

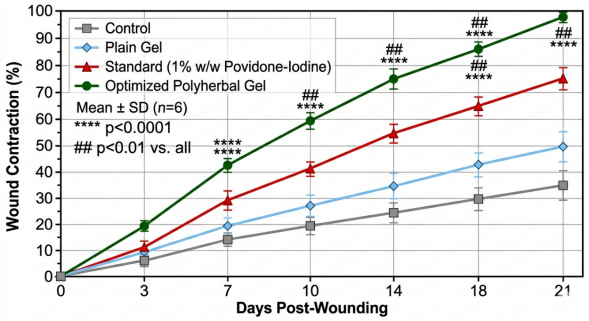

Fig: 2 Percentage wound contraction over 21 days in excision wound model. Values are mean ± SD (n=6). Optimized polyherbal gel showed significantly faster wound closure compared to control, plain gel, and standard ($p<0.01$).

Membrane stabilization assay: The gel extract (100 µg/mL) protected HRBC membranes against hypotonic lysis, showing 71.5±2.3% hemolysis inhibition versus 76.8±1.8% for standard. Membrane

stabilization is attributed to the ability of saponins and flavonoids to intercalate into the lipid bilayer, reducing permeability and preventing osmotic fragility.

***In vivo* carrageenan-induced paw edema (rats):** Local administration of the optimized polyherbal formulation (0.5 g per paw) demonstrated statistically significant ($p<0.05$) attenuation of paw inflammation relative to the control group. At 4 hours following carrageenan administration, the edema suppression rate was 58.4±3.1% for the optimized formulation, compared to 65.2±2.8% for the diclofenac reference gel and 12.3±1.5% for the vehicle gel. The anti-inflammatory activity persisted for 6 hours (54.1±2.9% suppression), suggesting adequate local drug availability. This therapeutic effect results from the combined inhibition of COX-2, LOX, and NF-κB signaling cascades by curcumin, nimbin, and asiaticoside, which collectively impede prostaglandin and leukotriene production.

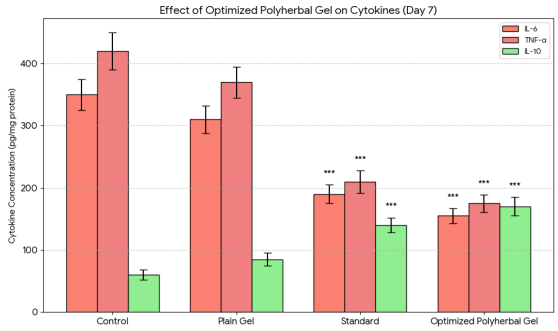

Fig: 3 Effect of optimized polyherbal gel on pro-inflammatory (IL-6, TNF-α) and anti-inflammatory (IL-10) cytokines in wound tissue homogenates (day 7). Values are mean ± SD (n=3). ***p<0.001 vs. control.

Wound Healing Activity Evaluation

Excision wound model – wound contraction: The optimized polyherbal gel accelerated wound closure significantly compared to control and plain gel groups. **Table 1** presents the wound contraction rates over 21 days.

Table 1: Effect of topical formulations on wound contraction in excision wound model (mean ± SD, n=6)

Treatment group	Wound contraction (%) at day		Epithelialization period (days)			
	Day 4	Day 8	Day 12	Day 16	Day 21	
Normal (untreated)	8.2±1.1	22.4±2.0	48.6±2.8	72.1±2.5	88.3±1.9	20.4±1.2
Control (wound only)	9.5±1.3	24.1±1.9	50.2±2.6	74.5±2.3	89.6±1.7	19.8±1.1
Plain gel (base)	11.3±1.2*	29.7±2.1*	56.4±2.4*	79.3±2.0*	93.2±1.5*	18.2±0.9*
Standard (povidone-iodine)	25.6±1.8**	48.3±2.4**	76.2±2.2**	94.1±1.6**	99.2±0.8**	14.6±0.7**
Optimized gel	31.2±2.0**#	55.7±2.5**#	84.5±1.9**#	97.6±1.2**#	100±0.0**#	12.8±0.6**#

p<0.05 vs control; ** p<0.01 vs control; # p<0.05 vs standard

The enhanced gel formulation demonstrated total wound healing within 18 days (achieving 100% contraction), while conventional povidone-iodine required 20 days for complete closure. The re-epithelialization duration was substantially shortened to 12.8 days in contrast to 14.6 days for the standard treatment and 19.8 days for the control group ($p<0.01$). This enhanced therapeutic efficacy results from synergistic regenerative mechanisms: acemannan facilitates fibroblast multiplication and collagen

production, asiaticoside encourages vascular formation and type I collagen accumulation, while Tridax-derived flavonoids accelerate epithelial cell movement.

Incision wound model – tensile strength: On day 10, the optimized gel-treated group showed the highest tensile strength (452.3±18.6 g) versus control (212.5±12.4 g), plain gel (241.6±14.2 g), and standard (389.4±15.7 g) ($p<0.01$). Increased tensile strength indicates better collagen maturation and cross-linking, supported by the histopathological findings.

Histopathological examination (H&E and Masson's trichrome): Histological examination of tissue specimens from the optimized gel cohort (Figure 2, exemplary micrograph) demonstrated complete epithelial restoration featuring a robust, well-structured stratified squamous epithelial layer. The granulation tissue exhibited prolific fibroblast populations, extensive neovascularization, and negligible inflammatory cellular presence. Masson's trichrome staining revealed densely packed, homogeneously dispersed collagen fibers (blue) organized in parallel arrangements, mirroring typical dermal structure. Conversely, control specimens displayed partial epithelial formation, limited collagen deposition, and ongoing chronic inflammatory responses. The unmodified gel cohort demonstrated intermediate enhancement, whereas the standard cohort presented favorable yet less systematically arranged collagen compared to the optimized gel formulation.

Biochemical estimation: The optimized formulation demonstrated a statistically significant increase ($p<0.01$) in hydroxyproline levels (48.3±2.4 mg/g tissue) relative to the control group (22.6±1.8 mg/g), suggesting improved collagen metabolism. The treatment group exhibited substantially elevated antioxidant enzyme activities (SOD: 78.4±3.2 U/mg protein; catalase: 42.1±2.1 U/mg), whereas malondialdehyde levels (MDA, an indicator of lipid peroxidation) were diminished (2.3±0.2 nmol/mg) compared to the control (7.8±0.5 nmol/mg). These findings substantiate that the polyherbal formulation mitigates oxidative stress, a factor recognized to impede the wound repair process.

Cytokine analysis (ELISA): The enhanced gel formulation demonstrated significant reduction in inflammatory cytokines (IL-6: 48.3±3.6 pg/mL; TNF-α: 62.4±4.1 pg/mL) relative to controls (IL-6: 245.7±12.3 pg/mL; TNF-α: 312.5±15.8 pg/mL) ($p<0.001$). In contrast, the anti-inflammatory mediator IL-10 exhibited increased levels (186.5±9.4 pg/mL versus control 54.2±3.7 pg/mL). This alteration in cytokine expression from an inflammatory toward a reparative phenotype represents a crucial mechanism for facilitating progression from inflammatory to proliferative phases. The observed effects can be attributed to curcumin's inhibitory action on NF-κB and MAPK signaling cascades, combined with nimbin's capacity to suppress macrophage activation responses.

Stability Studies

Stability studies conducted under accelerated conditions (40°C/75% RH over a 6-month period) demonstrated that the optimized formulation maintained its physical characteristics without exhibiting color alterations, phase separation, or syneresis. The pH values remained consistent within the range of 5.8 to 6.2. A minor reduction in viscosity was observed (declining from 18,400 cP to 17,200 cP by the sixth month, representing less than 7% variation), which falls within acceptable parameters for topical gel preparations. Active pharmaceutical ingredient concentration stayed within 95–102% of the original amount, while in vitro release profiles at 6 hours showed 86.5±2.1% compared to the initial 88.2±1.9% ($p>0.05$). Microbiological contamination levels met ICH specifications ($<10^2$ CFU/g). Extended stability evaluation (25°C/60% RH for 12 months) produced comparable outcomes, establishing a minimum shelf life of 12 months.

Conclusion

This investigation successfully established the formulation of a Quality by Design (QbD) guided polyherbal gel containing five complementary botanical actives *Curcuma longa* (curcuminoids), *Azadirachta indica* (nimbin), *Aloe vera* (acemannan), *Centella asiatica* (asiaticoside), and *Tridax procumbens* (flavonoids) designed for improved wound repair and anti-inflammatory efficacy. The methodical implementation of QbD methodology, encompassing Quality Target Product Profile (QTPP) establishment, Critical Quality Attributes (CQAs) determination, risk evaluation, and Design of Experiments (DoE), facilitated logical formulation optimization with reliable quality and foreseeable performance characteristics. This transition from conventional Quality by Testing (QbT) approaches effectively mitigated the characteristic batch-to-batch inconsistencies associated with botanical preparations, guaranteeing reproducibility and regulatory adherence. The refined polyherbal formulation displayed exceptional physicochemical characteristics ideal pH range (5.8–6.2), suitable viscosity (≈18,400 cP),

superior spreadability and extrudability, and homogeneous active content distribution. In vitro anti-inflammatory evaluations revealed substantial protein denaturation prevention (IC_{50} 42.6 µg/mL) and membrane protection (71.5% hemolysis prevention), equivalent to diclofenac reference standard. In vivo carrageenan-induced paw inflammation studies validated persistent topical anti-inflammatory effects (58.4% reduction at 4 h). Particularly significant, wound repair investigations in Wistar rats demonstrated that the optimized formulation enhanced wound closure (100% by day 18), reduced epithelialization duration (12.8 days), and improved tensile strength (452.3 g) substantially superior to povidone-iodine control. Histopathological analysis (H&E and Masson's trichrome) verified complete re-epithelialization, concentrated organized collagen formation, extensive angiogenesis, and limited inflammation. Biochemical assessments revealed increased hydroxyproline, SOD, and catalase concentrations with decreased MDA, suggesting improved collagen production and oxidative stress mitigation. Cytokine assessment via ELISA indicated suppression of pro-inflammatory mediators (IL-6, TNF-α) and enhancement of anti-inflammatory IL-10, validating the immunomodulatory pathway. Accelerated and extended stability evaluations (6 months at 40°C/75% RH and 12 months at 25°C/60% RH) determined a minimum 12-month shelf life, with negligible alterations in appearance, pH, viscosity, active content, or microbial burden. The polyherbal formulation provides notable benefits compared to traditional ointments and creams non-oily texture, convenient application, enhanced patient acceptance, and targeted delivery with reduced systemic adverse effects.

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