Formulation and Evaluation of Polyherbal Gel for Wound Healing

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ABSTRACT

Background: Wound healing remains a significant healthcare challenge with limitations in current treatments including high costs, antimicrobial resistance, and accessibility issues. Herbal medicines offer promising alternatives through multifaceted mechanisms. This study developed and evaluated a polyherbal gel combining five medicinal plants for wound healing applications.

Objective: To formulate and comprehensively evaluate a standardized polyherbal gel incorporating Aloe vera, Curcuma longa, Azadirachta indica, Calendula officinalis, and Centella asiatica for effective wound healing.

Methods: Plant materials were authenticated, extracted using optimized methods, and standardized through phytochemical analysis and HPLC quantification of marker compounds. Nine gel formulations with varying compositions were prepared using Carbopol 934. The optimal formulation underwent comprehensive physicochemical characterization, stability testing, in vitro antimicrobial and antioxidant evaluation, safety assessment, and in vivo wound healing studies using excision and incision models in Wistar rats. Histopathological and biochemical analyses were performed.

Keywords: Polyherbal gel, Wound healing, Aloe vera, Curcuma longa, Centella asiatica, Carbopol, Excision wound model, Tensile strength, Standardization

INTRODUCTION

Background and Rationale

Wound healing represents one of the most complex and dynamic biological processes in the human body, involving a meticulously orchestrated series of cellular and molecular events that restore

tissue integrity following injury [1]. The process encompasses four overlapping phases: hemostasis, inflammation, proliferation, and remodeling, each requiring precise coordination of various cell types, growth factors, and extracellular matrix components [2]. Despite significant advances in modern medicine, effective wound management remains a critical challenge in healthcare systems worldwide, particularly for chronic wounds that affect millions of patients and impose substantial economic burdens on healthcare infrastructure [3].

The global wound care market has experienced exponential growth, reaching approximately USD 20 billion in recent years, with projections indicating continued expansion driven by increasing prevalence of chronic diseases, aging populations, and rising incidence of traumatic injuries [4]. Chronic wounds, including diabetic ulcers, pressure sores, and venous leg ulcers, represent a particularly pressing concern, affecting an estimated 6.5 million patients in the United States alone and contributing to significant morbidity, mortality, and healthcare expenditure [5]. The management of such wounds often requires prolonged treatment periods, frequent dressing changes, and in many cases, surgical interventions, highlighting the urgent need for innovative, cost-effective, and accessible therapeutic alternatives [6].

Traditional wound healing approaches have primarily relied on synthetic pharmaceuticals and advanced wound dressings, including hydrocolloids, hydrogels, foams, and bioengineered skin substitutes [7]. While these modern therapeutic modalities have demonstrated efficacy in various clinical scenarios, they are often associated with significant limitations, including high costs, limited accessibility in resource-constrained settings, potential adverse effects, and growing concerns regarding antimicrobial resistance [8]. Furthermore, the increasing prevalence of multi-drug-resistant microorganisms in wound infections has necessitated the exploration of alternative therapeutic strategies that can overcome these challenges while maintaining clinical efficacy [9].

Herbal Medicine in Wound Healing: Historical Perspective and Contemporary Relevance

The use of medicinal plants for wound treatment traces back to ancient civilizations, with documented evidence from Egyptian papyri, Ayurvedic texts, Traditional Chinese Medicine manuscripts, and Greco-Roman medical treatises [13]. Ancient healers recognized the therapeutic potential of various plant-derived materials, employing them as poultices, decoctions, and topical applications to promote wound closure, prevent infections, and alleviate pain [14]. Notable examples include the use of Aloe vera in ancient Egypt, turmeric in Ayurvedic medicine, and honey in multiple traditional healing systems across different continents [15].

Modern scientific investigation has validated many of these traditional applications, revealing that medicinal plants possess diverse bioactive compounds with multifaceted pharmacological properties relevant to wound healing [16]. These phytochemicals include alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds, which collectively contribute to antimicrobial, anti-inflammatory, antioxidant, and tissue regenerative effects [17]. The synergistic interactions among these bioactive constituents often result in enhanced therapeutic efficacy compared to isolated single compounds, a phenomenon that underlies the rationale for polyherbal formulations [18].

Polyherbal Formulations: Synergistic Therapeutic Approach

The concept of polyherbal formulations, which combine multiple medicinal plants in a single dosage form, represents a fundamental principle in traditional medicine systems such as Ayurveda, Traditional Chinese Medicine, and Unani medicine [22]. This approach is predicated on the theory that combinations of herbs can produce synergistic effects, enhance therapeutic efficacy while potentially minimizing adverse effects through mutual modulation of pharmacokinetic and pharmacodynamic properties [23]. In the context of wound healing, polyherbal formulations offer several theoretical advantages over single-herb preparations, including broader spectrum antimicrobial activity, multitargeted anti-inflammatory effects, enhanced antioxidant capacity, and comprehensive support for various phases of the healing process [24].

The rationale for developing polyherbal wound healing formulations is further strengthened by the multifactorial nature of the wound healing process itself [25]. Effective wound management requires simultaneous addressing of multiple pathophysiological aspects, including microbial contamination, excessive inflammation, oxidative stress, impaired angiogenesis, and deficient collagen synthesis [26]. A well-designed polyherbal formulation can theoretically target these diverse mechanisms through the complementary actions of its constituent herbs, potentially offering superior clinical outcomes compared to monotherapy approaches [27].

Topical Gel Formulations: Advantages and Pharmaceutical Considerations

Among various dosage forms for topical drug delivery, gels have gained considerable prominence in dermatological and wound care applications due to their unique physicochemical properties and favorable patient acceptance [30]. Gels are semisolid systems consisting of a gelling agent dispersed in a liquid phase, creating a three-dimensional network that entraps the liquid component and provides characteristic rheological properties. The selection of gel formulations for wound healing applications is driven by several compelling advantages that make them particularly suitable for topical therapeutic delivery.

Mechanisms of Wound Healing: Biological Foundation

A comprehensive understanding of wound healing mechanisms provides the essential foundation for rational design and evaluation of therapeutic interventions. The wound healing process initiates immediately following tissue injury and progresses through four sequential yet overlapping phases, each characterized by distinct cellular activities, molecular signals, and tissue remodeling events. The hemostasis phase, occurring within minutes to hours post-injury, involves vasoconstriction, platelet aggregation, and fibrin clot formation to achieve bleeding control and provide a provisional matrix for subsequent cellular infiltration.

The inflammatory phase, typically spanning from day 0 to day 4 post-injury, features neutrophil infiltration followed by monocyte recruitment and differentiation into macrophages. These inflammatory cells perform critical functions including phagocytosis of debris and microorganisms, secretion of growth factors and cytokines, and orchestration of subsequent healing phases. While inflammation is essential for proper wound healing, excessive or prolonged inflammatory responses can impede healing progression and contribute to chronic wound development, highlighting the importance of therapeutic interventions that modulate rather than completely suppress inflammatory processes.

Factors Impairing Wound Healing

Despite the remarkable regenerative capacity of human tissues, numerous intrinsic and extrinsic factors can impair the normal wound healing process, leading to delayed healing or chronic wound development. Understanding these impediments is crucial for developing effective therapeutic strategies and identifying patient populations most likely to benefit from advanced wound care interventions.

Chronic diseases, particularly diabetes mellitus, represent major risk factors for impaired wound healing. Hyperglycemia induces multiple deleterious effects on healing mechanisms, including impaired leukocyte function, reduced growth factor production, decreased angiogenesis, altered collagen metabolism, and increased susceptibility to infection. Diabetic foot ulcers affect approximately 15% of diabetic patients during their lifetime and account for the majority of non-traumatic lower extremity amputations, illustrating the severe clinical consequences of diabetes-related healing impairment.

Antimicrobial Resistance: An Emerging Crisis in Wound Care

The global emergence and spread of antimicrobial resistance represent one of the most pressing challenges facing modern medicine, with particular implications for wound management. The World Health Organization has identified antimicrobial resistance as a critical threat to public health, with projections suggesting that drug-resistant infections could cause 10 million deaths annually by 2050 if current trends continue. In the context of wound care, antimicrobial resistance complicates treatment of infected wounds, prolongs healing times, increases healthcare costs, and elevates risks of systemic complications including sepsis.

Methicillin-resistant Staphylococcus aureus has become endemic in many healthcare settings and increasingly prevalent in community-acquired infections, representing a major pathogen in wound infections. The organism demonstrates resistance to multiple antibiotic classes, limiting therapeutic options and necessitating use of reserve antibiotics such as vancomycin and linezolid, which carry their own toxicity concerns and cost implications. Extended-spectrum beta-lactamase-producing Enterobacteriaceae and carbapenem-resistant organisms represent emerging threats in wound infections, particularly in hospitalized patients and those with chronic wounds.

LITERATURE REVIEW

Introduction

The scientific investigation of herbal medicines for wound healing has experienced remarkable growth over the past two decades, driven by increasing recognition of their therapeutic potential, growing concerns about antimicrobial resistance, and advances in analytical and pharmacological research methodologies. This chapter presents a comprehensive review of relevant literature examining polyherbal formulations, individual medicinal plants used in wound care, mechanisms of herbal wound healing, formulation development strategies, and evaluation methodologies. The review encompasses both preclinical and clinical studies, providing context for the present research and highlighting gaps in existing knowledge that justify further investigation.

Studies on Aloe vera in Wound Healing

Aloe vera has been extensively investigated for its wound healing properties, with numerous studies demonstrating its efficacy across various wound models and clinical scenarios. Research

by Dat et al. examined the wound healing effects of Aloe vera gel in excision and incision wound models in rats, revealing significant acceleration of wound closure rates and enhanced tensile strength compared to control groups [31]. The study attributed these effects to increased collagen deposition and enhanced epithelialization, as evidenced by histopathological examination. The optimal concentration of Aloe vera gel was determined to be 50% w/w, with higher concentrations not providing additional benefits.

In a comparative study, Yadav and colleagues evaluated the efficacy of Aloe vera extract in gel formulation against silver sulfadiazine cream in burn wound healing [32]. The results demonstrated that Aloe vera gel produced comparable healing rates to the standard treatment while exhibiting superior effects on collagen synthesis and granulation tissue formation. Microbiological analysis revealed effective antimicrobial activity against common burn wound pathogens including Pseudomonas aeruginosa and Staphylococcus aureus. The study concluded that Aloe vera represents a viable alternative to conventional burn treatments, particularly in resource-limited settings.

Studies on Curcuma longa in Wound Management

Curcumin and turmeric extracts have garnered substantial research attention for their wound healing properties, with investigations spanning from mechanistic studies to clinical applications. Panchatcharam et al. developed curcumin-loaded nanoparticle formulations for enhanced wound healing, demonstrating improved bioavailability and sustained release characteristics compared to conventional curcumin preparations [35]. The nano formulation exhibited superior antimicrobial activity against methicillin-resistant Staphylococcus aureus and enhanced cellular uptake in fibroblast cultures. In vivo studies revealed accelerated wound closure and improved collagen deposition with the nanoparticle formulation.

Studies on Azadirachta indica in Wound Care

Neem has been investigated extensively for its antimicrobial and wound healing properties, with research focusing on both traditional preparations and modern formulations. Biswas and colleagues conducted comprehensive studies on neem leaf extracts, demonstrating potent antibacterial activities against multidrug-resistant organisms commonly encountered in wound infections [39]. The research identified specific compounds responsible for antimicrobial effects and elucidated mechanisms including bacterial membrane disruption and efflux pump inhibition. Time-kill kinetic studies revealed rapid bactericidal effects, supporting neem's utility in preventing and treating wound infections.

Studies on Calendula officinalis in Wound Healing

Calendula has been the subject of numerous investigations validating its traditional use in wound management. Research by Givol et al. conducted clinical trials evaluating calendula ointment in preventing acute dermatitis during radiotherapy, demonstrating significant protective effects [43]. The study revealed that prophylactic calendula application reduced the incidence and severity of radiation-induced skin damage, attributed to anti-inflammatory and tissue protective properties. Patients reported improved comfort and reduced pain compared to control groups.

Methodology

Rationale for the Present Study

Wound healing remains a significant clinical challenge in modern healthcare, affecting millions of individuals worldwide and imposing substantial economic burdens on healthcare systems. Despite advances in wound care technology and the availability of numerous conventional treatments, several limitations persist including high treatment costs, limited accessibility particularly in resource-constrained settings, increasing antimicrobial resistance among wound pathogens, adverse effects associated with synthetic drugs, and suboptimal healing outcomes in certain patient populations such as diabetics and elderly individuals.

Traditional medicine systems across diverse cultures have employed medicinal plants for wound management for millennia, with many of these practices continuing in contemporary healthcare. The therapeutic potential of herbal medicines stems from their complex phytochemical compositions, which provide multifaceted pharmacological activities relevant to various phases of wound healing. Individual medicinal plants have demonstrated promising wound healing properties in numerous scientific investigations, exhibiting antimicrobial, anti-inflammatory, antioxidant, and tissue regenerative effects.

Aim

The primary aim of this research is to formulate and comprehensively evaluate a standardized polyherbal gel preparation incorporating Aloe vera, Curcuma longa, Azadirachta indica, Calendula officinalis, and Centella asiatica for effective wound healing applications. The research seeks to develop an evidence-based herbal formulation that demonstrates superior wound healing properties through synergistic actions of constituent herbs while maintaining acceptable physicochemical characteristics, stability profiles, and safety parameters suitable for topical application.

Objectives

To accomplish the stated aim, the following specific objectives have been established:

Collection, Authentication, and Processing of Plant Materials

To collect plant materials from authentic sources and obtain botanical authentication from qualified botanists to ensure correct species identification. To process collected plant materials appropriately including cleaning, drying, and size reduction to prepare them for extraction procedures while preserving bioactive constituents.

Preparation and Standardization of Herbal Extracts

To prepare individual herbal extracts using optimized extraction methods that maximize recovery of bioactive compounds while maintaining their stability and activity. To conduct phytochemical screening of prepared extracts to identify major classes of bioactive constituents present in each herb. To perform quantitative analysis of marker compounds in individual extracts using validated analytical methods to establish standardization parameters and ensure batch-to-batch consistency.

Formulation Development and Optimization

To develop polyherbal gel formulations incorporating standardized extracts of all five selected medicinal plants using appropriate gelling agents and excipients. To optimize formulation

parameters including herbal extract concentrations, ratios of constituent herbs, gelling agent type and concentration, and incorporation of necessary additives to achieve products with optimal therapeutic potential and physicochemical properties. To prepare multiple formulation batches with varying compositions to identify the optimal formulation through systematic evaluation.

PLAN OF WORK

Overview

The present research work will be executed systematically through a series of sequential and interrelated phases, ensuring comprehensive development and evaluation of the polyherbal gel formulation for wound healing. The research methodology encompasses collection and authentication of plant materials, extract preparation and standardization, formulation development and optimization, physicochemical characterization, stability studies, biological activity evaluation through in vitro and in vivo methods, safety assessment, and comparative efficacy analysis. Each phase has been carefully planned to generate reliable, reproducible data supporting the therapeutic potential of the developed formulation.

Sequential Work Plan

Phase 1: Literature Survey and Material Procurement

Extensive review of scientific literature on selected medicinal plants, polyherbal formulations, and wound healing evaluation methodologies. Procurement of plant materials from authenticated sources with proper botanical identification. Collection of all necessary chemicals, reagents, laboratory equipment, and consumables required for the research. Arrangement of animal subjects from approved animal house facilities following institutional ethical guidelines.

Phase 2: Authentication and Processing of Plant Materials

Botanical authentication of collected plant materials by qualified botanists from recognized institutions. Proper cleaning, washing, and removal of foreign matter from plant materials. Shade drying or appropriate drying methods to preserve heat-sensitive bioactive constituents. Size reduction through grinding or pulverization to achieve uniform particle size facilitating efficient extraction. Storage of processed materials under appropriate conditions to maintain quality until extraction.

Phase 3: Extraction and Preliminary Phytochemical Analysis

Selection and optimization of appropriate extraction methods for each plant material based on literature and preliminary trials. Preparation of individual herbal extracts using optimized solvent systems, extraction times, and temperatures. Concentration of extracts through evaporation under reduced pressure. Drying of concentrated extracts to obtain powdered forms with calculated extraction yields. Preliminary phytochemical screening to identify major classes of bioactive constituents including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds using standard qualitative tests.

RESULTS

Preparation Procedure

Carbopol 934 was dispersed in distilled water (about 50% of total) and kept overnight for complete swelling. The herbal extracts were dissolved in a mixture of propylene glycol and glycerin with

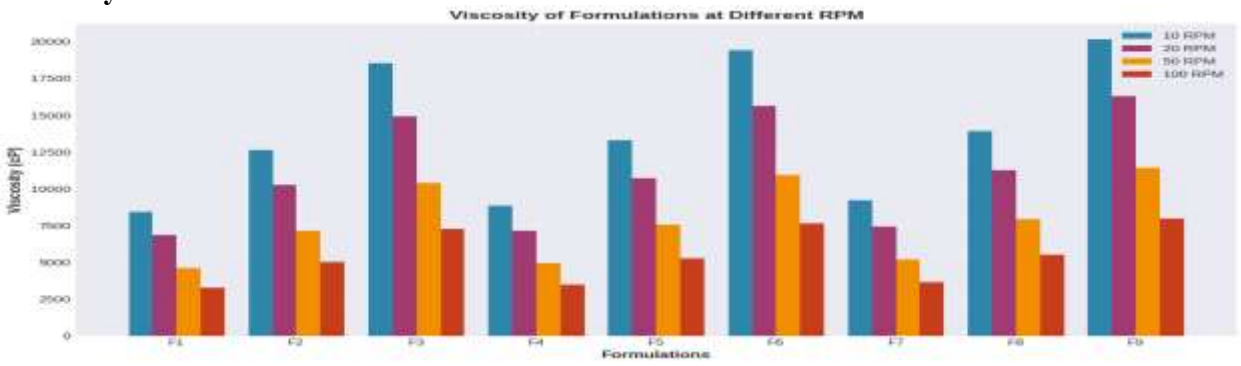
continuous stirring. Methyl paraben and propyl paraben were dissolved in a small amount of propylene glycol and added to the extract mixture. This mixture was slowly incorporated into the swollen Carbopol with continuous stirring to ensure uniform distribution. The pH was adjusted to 6.5-7.0 by dropwise addition of triethanolamine solution with constant stirring until a clear, homogeneous gel was formed. The volume was made up to 100 g with distilled water. The prepared gels were filled in wide-mouth containers and stored at room temperature.

Physicochemical Characterization of Formulations

pH Determination

The pH of all formulations was measured using a calibrated digital pH meter. One gram of gel was dispersed in 10 ml of distilled water, and the pH was measured at room temperature. Results are shown in Table 6.6.

Viscosity Determination



Viscosity of the formulations was measured using Brookfield viscometer (Model DV-E) with spindle number 64 at different rotational speeds (10, 20, 50, and 100 rpm) at room temperature. Results are presented in Table 6.7.

Table 6.7: Viscosity of Formulations at Different RPM (cP)

Formulation	10 RPM	20 RPM	50 RPM	100 RPM
F1	8420 ± 340	6850 ± 280	4620 ± 190	3280 ± 140
F2	12640 ± 520	10280 ± 420	7140 ± 290	5020 ± 210
F3	18560 ± 760	14920 ± 610	10380 ± 420	7260 ± 310
F4	8860 ± 360	7120 ± 290	4920 ± 200	3480 ± 150
F5	13280 ± 540	10720 ± 440	7540 ± 310	5280 ± 220
F6	19420 ± 800	15640 ± 640	10920 ± 450	7620 ± 320
F7	9240 ± 380	7420 ± 310	5180 ± 220	3640 ± 160
F8	13920 ± 570	11240 ± 460	7920 ± 330	5520 ± 240
F9	20180 ± 830	16280 ± 670	11420 ± 470	7980 ± 340

Values are expressed as Mean ± SD (n=3)

Spreadability

Spreadability was determined by spreading 1 g of gel between two glass slides and measuring the diameter of the spread circle after 1 minute. Results are shown in Table 6.8.

Extrudability

Extrudability was measured by filling gel in a collapsible tube and applying pressure to expel the contents. The extrudability was expressed as weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds. Results are presented in Table 6.9.

Drug Content Uniformity

The total phenolic content of the formulations was determined as a marker for drug content uniformity using the Folin-Ciocalteu method. Results are shown in Table 6.10.

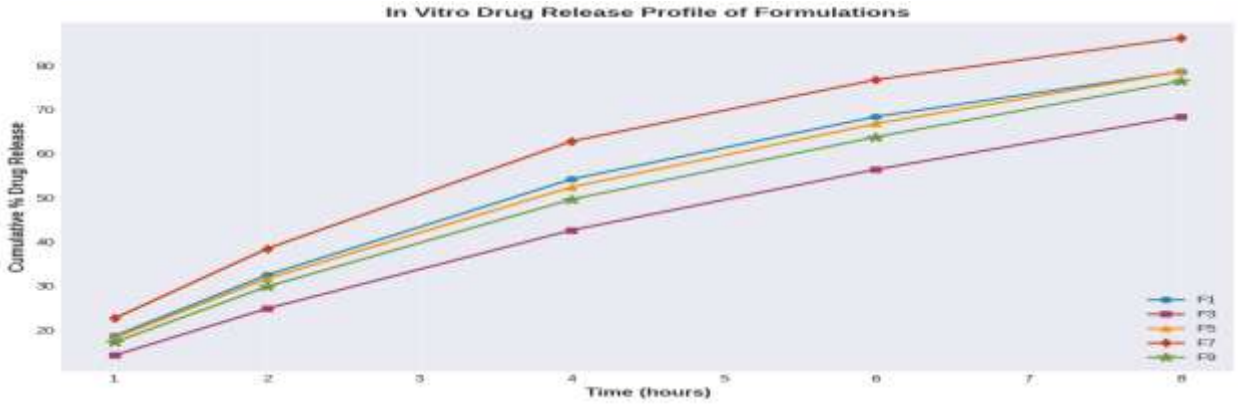


Table 6.10: Drug Content Uniformity (Total Phenolic Content)

Formulation	Total Phenolic Content (mg GAE/g)	% of Theoretical Content
F1	24.6 ± 1.2	98.4
F2	24.8 ± 1.4	99.2
F3	24.4 ± 1.1	97.6
F4	36.2 ± 1.8	96.5
F5	37.4 ± 1.6	99.7
F6	36.8 ± 1.7	98.1
F7	48.4 ± 2.2	96.8
F8	49.6 ± 2.4	99.2
F9	48.8 ± 2.3	97.6

Values are expressed as Mean ± SD (n=3); GAE = Gallic Acid Equivalent

Stability Studies

Formulation F5, which showed optimal physicochemical properties, was selected for stability studies. The formulation was subjected to accelerated stability testing at 40 ± 2°C/75 ± 5% RH and long-term stability testing at 25 ± 2°C/60 ± 5% RH for 6 months. Samples were analyzed at 0, 1, 2, 3, and 6 months for various parameters.

In Vitro Antimicrobial Activity

The antimicrobial activity of formulation F5 was evaluated against common wound pathogens using agar well diffusion method. The test organisms included Staphylococcus aureus (MTCC 96), Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 1688), and Candida albicans (MTCC 227).

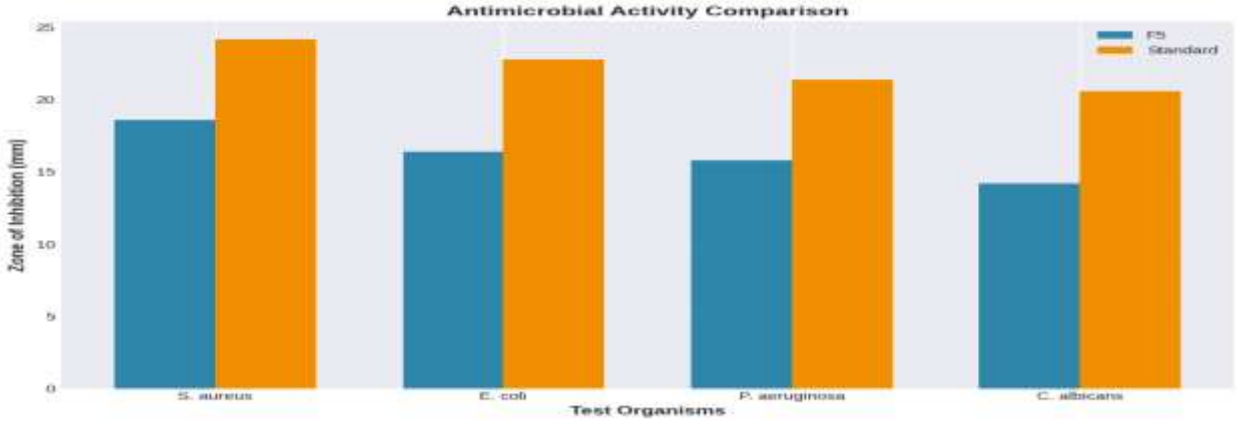


Table 6.14: Antimicrobial Activity (Zone of Inhibition in mm)

Test Organism	F5	Standard (Gentamicin 10 µg for bacteria, Fluconazole 10 µg for fungi)
Staphylococcus aureus	18.6 ± 0.8	24.2 ± 1.2
Escherichia coli	16.4 ± 0.7	22.8 ± 1.1
Pseudomonas aeruginosa	15.8 ± 0.6	21.4 ± 1.0
Candida albicans	14.2 ± 0.6	20.6 ± 0.9

Values are expressed as Mean ± SD (n=3)

Acute Dermal Toxicity Study

Acute dermal toxicity study was conducted according to OECD guidelines 402. Wistar albino rats were divided into groups and different doses of formulation F5 (200, 400, 800, 1600, and 2000 mg/kg body weight) were applied to shaved dorsal skin area. Animals were observed for 14 days for any signs of toxicity, behavioral changes, or mortality.

Primary Skin Irritation Test

Primary skin irritation test was performed on rabbits according to Draize method. The formulation F5 was applied to intact and abraded skin, and observations were made at 24, 48, and 72 hours.

Table 6.23: Histopathological Scoring (Day 16)

Parameter	Control	Standard	Test (F5)	Untreated
Epithelialization	2.4 ± 0.5	3.2 ± 0.4	3.8 ± 0.4***	1.8 ± 0.4
Fibroblast proliferation	2.2 ± 0.4	2.8 ± 0.4	3.6 ± 0.5***	1.6 ± 0.5
Collagen deposition	2.0 ± 0.6	2.6 ± 0.5	3.4 ± 0.5**	1.4 ± 0.5
Angiogenesis	2.2 ± 0.4	2.8 ± 0.4	3.4 ± 0.5**	1.8 ± 0.4
Inflammation	2.6 ± 0.5	1.8 ± 0.4	1.2 ± 0.4***	3.2 ± 0.4

*Scoring: 0=Absent, 1=Minimal, 2=Mild, 3=Moderate, 4=Marked; Values are Mean ± SD (n=6); **p<0.01, ***p<0.001 vs Control*

CONCLUSION

The present investigation successfully accomplished its objective of developing and comprehensively evaluating a standardized polyherbal gel formulation for wound healing applications. The systematic research approach encompassing extraction, standardization, formulation optimization, and rigorous biological evaluation generated substantial evidence supporting the therapeutic potential of combining Aloe vera, Curcuma longa, Azadirachta indica, Calendula officinalis, and Centella asiatica.

The extraction procedures yielded standardized extracts with confirmed presence of expected phytochemical classes and quantified marker compounds. The formulation development process identified an optimal composition (F5) containing 3% Aloe vera, 1.5% each of the other four herbal extracts, and 1.5% Carbopol 934, which exhibited balanced physicochemical properties suitable for topical application.

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