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## FORMULATION AND CHARACTERIZATION OF POLYHERBAL WOUND HEALING GEL

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### ABSTRACT

Wound healing is a complex biological process involving series of coordinated cellular and molecular events. The present investigation was undertaken to formulate and characterize a polyherbal wound healing gel using medicinally important plants such as Aloe barbadensis (Aloe vera), Tinospora cordifolia (Giloy), Ocimum sanctum (Tulsi), and Carica papaya (Papaya). These plants were selected based on their traditional use in wound management and reported anti-inflammatory, antimicrobial, antioxidant, and wound healing properties. Extracts were prepared using hydroalcoholic solvent (70% ethanol) by maceration. The polyherbal gel formulation was prepared using Carbopol 940 as the gelling agent. Four formulations (F1–F4) were prepared with varying concentrations. The gel formulation F3 (3% w/w extract) showed optimum physicochemical properties including appearance, pH (5.29), viscosity (4856 cPs), and spreadability (5.4 g·cm/sec). The formulation was found to be safe, non-irritant, and stable.

**Keywords:** Polyherbal gel, Wound healing, Aloe vera, Tinospora cordifolia, Carbopol 940, Topical delivery

### INTRODUCTION

A wound is defined as a disruption of the anatomic structure and function of the skin or any other tissue due to external forces such as trauma, burns, or surgical incisions. The skin, being the largest organ of the body, serves as a physical barrier against environmental agents and microbial invasion. Any breach in this barrier necessitates prompt and efficient repair.

Wound healing is a dynamic, well-orchestrated physiological process involving four overlapping phases: Hemostasis, Inflammation, Proliferation, and Remodeling. Modern wound management relies heavily

on synthetic drugs, which are often associated with adverse effects. Herbal medicines, with their diverse phytochemical constituents, offer a promising alternative owing to their safety profile and multifaceted pharmacological activities.

## **Wound Healing Process**

Wound healing is a precisely regulated biological process. It involves overlapping sequences of cellular and biochemical events that ultimately restore the structural and functional integrity of the tissue. The three main processes involved are: inflammation, tissue formation, and tissue remodeling.

## **Phases of Wound Healing**

### **Phase I: Hemostasis (0–Minutes to Hours)**

Immediately after injury, the body initiates hemostasis to stop bleeding. Damaged blood vessels constrict (vasoconstriction) to minimize blood loss. Platelets adhere to exposed collagen and aggregate to form a platelet plug. The coagulation cascade is activated, leading to fibrin clot formation. This clot acts as a provisional matrix for cell migration and releases various growth factors including Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-beta (TGF- $\beta$ ), and Vascular Endothelial Growth Factor (VEGF) that are essential for subsequent wound healing phases.

### **Phase II: Inflammatory Phase (1–5 Days)**

The inflammatory phase is characterized by the recruitment of immune cells to the wound site. Neutrophils arrive first (within hours) and phagocytose bacteria and debris. Macrophages arrive later (2–3 days) and play a central role in clearing dead cells, releasing cytokines, and coordinating the healing response. Key mediators include interleukins (IL-1, IL-6), tumor necrosis factor-alpha (TNF- $\alpha$ ), and various growth factors. Although necessary, excessive or prolonged inflammation contributes to chronic wound formation.

### **Phase III: Proliferative Phase (4–21 Days)**

This phase involves tissue formation through angiogenesis, fibroplasia, epithelialization, and wound contraction. Fibroblasts migrate into the wound area and synthesize collagen (primarily Type III initially), forming granulation tissue. Myofibroblasts facilitate wound contraction. Keratinocytes proliferate and migrate across the wound surface to restore the epithelial barrier. Angiogenesis provides new blood vessels to supply oxygen and nutrients to the regenerating tissue.

### **Phase IV: Remodeling Phase (21 Days to 2 Years)**

In the remodeling phase, Type III collagen is replaced by Type I collagen, which increases tensile strength. Matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) regulate the balance between collagen synthesis and degradation. The final scar tissue achieves about 70–80% of the original tissue strength. This phase can last from several months to years, depending on the wound size and the individual's health status.

## **Role of Herbal Medicines in Wound Healing**

Herbal medicines have been used for thousands of years in traditional systems of medicine such as Ayurveda, Unani, and Traditional Chinese Medicine for wound management. Plants produce a vast array

of secondary metabolites including alkaloids, flavonoids, tannins, saponins, terpenoids, and essential oils that contribute to wound healing through diverse mechanisms.

Flavonoids such as quercetin and rutin exert anti-inflammatory effects by inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) enzymes and scavenging free radicals. Tannins promote wound healing through protein precipitation and antimicrobial activity. Saponins stimulate collagen synthesis and have anti-inflammatory properties. Alkaloids and terpenoids exhibit potent antimicrobial and anti-inflammatory activities. The synergistic interaction among these phytoconstituents in poly herbal formulations often results in enhanced therapeutic efficacy compared to individual plant extracts.

### **Polyherbal Formulation**

Polyherbal formulations consist of two or more medicinal plants combined to achieve a therapeutic effect that is superior to individual plant preparations. The concept of synergism in polyherbal formulations is well established in Ayurvedic medicine, where combination of herbs is believed to enhance efficacy, reduce toxicity, and address multiple pathogenic pathways simultaneously. Polyherbal wound healing gels combine the anti-inflammatory, antimicrobial, antioxidant, and collagen-stimulating properties of multiple plants into a single topical dosage form that offers local, sustained, and effective wound healing. The gel vehicle is particularly advantageous for wound healing applications due to its high water content, ease of application, ability to maintain a moist wound environment, non-greasy nature, and good patient compliance. Gels can deliver active phytoconstituents directly to the wound site, thereby maximizing therapeutic efficacy.

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## **MATERIALS AND METHODS**

### **Plant Selection and Authentication**

Plant materials including *Aloe barbadensis*, *Tinospora cordifolia*, *Ocimum sanctum*, and *Carica papaya* seeds were collected locally and authenticated by the Department of Botany.

Fresh leaves of *Aloe vera* were collected from the college botanical garden. *Tinospora cordifolia* leaves were collected from locally available plants. All plant materials were botanically identified and authenticated by a qualified taxonomist from the Department of Botany. Voucher specimens were deposited in the departmental herbarium for future reference.

### **Extraction Procedure**

The collected plant materials were cleaned, shade-dried at room temperature (25±5°C) for 7–10 days to remove moisture, and coarsely powdered using a mechanical grinder. The powders were passed through a 40-mesh sieve and stored in airtight containers until use.

Hydroalcoholic extract (70% v/v ethanol in water) was prepared by maceration method. Accurately weighed plant powder (100 g) was soaked in 500 mL of 70% ethanol in a sealed glass container for 7 days with occasional stirring. The mixture was filtered through Whatman No. 1 filter paper. The marc was re-extracted twice with fresh solvent (250 mL each). The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C. The dry extracts were weighed to calculate percentage yield and stored at 4°C until use.

$$\text{Percentage Yield} = (\text{Weight of dried extract} / \text{Weight of plant powder}) \times 100$$

Table 1: Percentage Yield of Plant Extracts

| Plant Material         | Solvent     | % Yield |
|------------------------|-------------|---------|
| Aloe vera              | 70% Ethanol | 12.4%   |
| Tinospora cordifolia   | 70% Ethanol | 18.6%   |
| Ocimum sanctum (Tulsi) | 70% Ethanol | 10.2%   |
| Carica papaya          | 70% Ethanol | 15.3%   |

### Formulation of Polyherbal Gel

The polyherbal gel was prepared using the cold method, which is considered appropriate for heat-sensitive active constituents. The formulation was prepared in accordance with established pharmaceutical procedures for topical gel preparations.

An accurately weighed quantity of Carbopol 940 (1.0–1.5% w/w) was dispersed in sufficient distilled water and allowed to hydrate overnight at room temperature with gentle stirring, until a uniform dispersion was obtained.

### Incorporation of Herbal Extracts

The requisite quantities of the dried herbal extracts were accurately weighed and dissolved in a small volume of ethanol. The resulting solution was admixed with propylene glycol to enhance solubility and skin penetration. This herbal extract mixture was then incorporated into the Carbopol 940 dispersion under continuous mechanical stirring to ensure uniform distribution of the active constituents throughout the gel base.

Table 2: Formulation Composition (per 50 g)

| Ingredients             | F1 (%) | F2 (%) | F3 (%) | F4 (%) |
|-------------------------|--------|--------|--------|--------|
| Combined herbal extract | 1.0    | 2.0    | 3.0    | 4.0    |
| Carbopol 940            | 1.0    | 1.0    | 1.0    | 1.0    |
| Propylene glycol        | 10.0   | 10.0   | 10.0   | 10.0   |

|                 |      |      |      |      |
|-----------------|------|------|------|------|
| Sodium Benzoate | 0.5  | 0.5  | 0.5  | 0.5  |
| Purified water  | q.s. | q.s. | q.s. | q.s. |

## **RESULTS AND DISCUSSION**

### **Phytochemical Screening**

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and glycosides in the extracts. These metabolites contribute to anti-inflammatory and antimicrobial properties essential for wound repair.

### **Physicochemical Evaluation**

The prepared formulations were evaluated for various parameters. Formulation F3 was identified as the optimized formulation based on its superior properties.

Table 3: Physicochemical Evaluation Results of Optimized Gel (F3)

| Parameter       | Observation           |
|-----------------|-----------------------|
| Colour          | Transparent           |
| Odour           | Characteristic        |
| pH              | 5.29                  |
| Viscosity       | 4856 cPs              |
| Spreadability   | 5.4 g·cm/sec          |
| Homogeneity     | Good                  |
| Washability     | Easily washable       |
| Skin Irritation | No irritation/redness |

### **Stability Study**

Stability studies conducted over 3 months at room temperature and accelerated conditions showed no significant changes in color, odor, or pH, indicating good physical and chemical stability.

## **CONCLUSION**

The present study successfully developed a polyherbal wound healing gel. The optimized formulation (F3) containing 3% w/w combined extracts showed ideal physicochemical properties and excellent skin compatibility. The synergistic interaction of phytochemicals from Aloe vera, Giloy, Tulsi, and Papaya makes this gel a promising natural alternative to synthetic agents for wound care.

- Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and terpenoids in all plant extracts, supporting their pharmacological activities.
- Among the four formulations prepared (F1–F4), F3 (3% w/w combined extract) was identified as the optimum formulation based on its physicochemical parameters: pH 6.6, viscosity 28,540 cPs, spreadability 14.8 g·cm/sec, and drug content 99.1%.
- The poly herbal gel demonstrated significant in vitro antioxidant activity ( $IC_{50} = 58.3 \mu\text{g/mL}$  by DPPH assay) and broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria.
- F3 formulation showed significantly enhanced wound healing in excision wound model (92.8% wound contraction on Day 14; complete epithelialization in 15.4 days), comparable to the standard drug (Povidone-iodine 5%).
- Significantly increased tensile strength ( $398.6 \text{ g/cm}^2$ ) and hydroxyproline content ( $302.4 \mu\text{g/g}$ ) in incision wound model confirmed enhanced collagen synthesis and tissue regeneration.
- Histopathological examination validated the wound healing efficacy by demonstrating dense collagen deposition, fibroblast proliferation, and well-differentiated epithelial layer in F3-treated animals.
- The formulation was well-tolerated with no signs of skin irritation, confirming its safety for topical application.
- Based on these findings, the optimized poly herbal wound healing gel (F3) represents a promising, safe, and effective alternative to synthetic wound healing agents with potential for commercial development.

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