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## Research Paper

# Development, Standardization and Stability Studies of Novel Ayurvedic Polyherbal Wound Healing Gel Formulation with Comparative Evaluation to Marketed Products

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## ABSTRACT

**Background:** Wound healing is a complex biological process that involves inflammation, cell proliferation, collagen synthesis, and tissue remodeling. Although several marketed topical formulations are available, their prolonged use may lead to adverse effects or delayed healing. Ayurvedic polyherbal formulations offer a promising alternative due to their synergistic therapeutic actions and improved safety profile.

**Objective:** The present study aimed to formulate, standardize, and comparatively evaluate a novel Ayurvedic polyherbal wound-healing gel containing *Catharanthus roseus*, *Chlorophytum borivilianum* (Safed Musli), and *Tinospora cordifolia* with selected marketed wound-healing formulations. **Methods:** Hydroalcoholic extracts of the selected medicinal plants were incorporated into a Carbopol-based gel. The formulated gel was evaluated for physicochemical properties including appearance, pH, viscosity, spread ability, extrudability, and drug content uniformity. Microbiological quality was assessed by total microbial load and absence of specified pathogens. Antimicrobial activity was evaluated against common wound-infecting microorganisms. **In vivo** wound-healing efficacy was assessed using excision and incision wound models by measuring wound contraction, epithelialization period, and tensile strength. Stability studies were conducted under accelerated conditions. **Results:** The novel polyherbal gel exhibited satisfactory physicochemical characteristics, acceptable microbial limits, and superior antimicrobial activity compared to marketed formulations. **In vivo** studies demonstrated significantly enhanced wound contraction, reduced epithelialization period, and increased tensile strength of healed tissue. Stability evaluation indicated no significant changes in formulation properties during the study period. **Conclusion:** The findings suggest that the novel Ayurvedic polyherbal wound-healing gel containing *Catharanthus roseus*, *Chlorophytum borivilianum*, and *Tinospora*

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cordifolia possesses improved wound-healing potential and stability compared to marketed products, supporting its use as a safe and effective herbal alternative for topical wound management.

## INTRODUCTION

Wound healing is a potent natural cycle that is essential for maintaining tissue integrity and preventing infections. Despite advances in clinical research, achieving optimal injury recovery remains difficult, necessitating creative restorative approaches. Due to their potential to heal injuries, natural remedies have often replaced conventional medicine, which has been developed over hundreds of years of precise data. Polyherbal formulations, which blend several herbs in a synergistic way, are becoming more and more popular as a promising way to improve the effectiveness of wound treatment(1).

It covers the historical background of herbal treatments in wound care across diverse cultures and lays the groundwork for understanding the reasoning for mixing different plants in therapeutic formulations. The focus on polyherbal gels stems from their potential advantages over single-spice medications, providing a complete approach to wound healing. As wound administration goes on being a worldwide wellness worry, this study endeavours to overcome any barrier between customary knowledge and present logical techniques. By exploring the development, design, and presentation of polyherbal gels (2) Additionally, this survey takes into account the challenges encountered during the development and evaluation of polyherbal gels, providing a fair assessment of the current state of research. Insights from ongoing clinical trials and human research that evaluate the importance of polyherbal gels in real-world scenarios offer a translational perspective(3).

### 1.1 Traditionally, medicinal herbs have been used to cure wounds.

Throughout history, people have utilized a variety of plant parts to cure and prevent a wide range of illnesses. In the past, plants were the primary source of medicinal treatments; these might be available as basic plant parts or in more complex forms like blends and crude extracts. Plants are still used to make a wide range of drugs that are effective in treating various illnesses. Most of these drugs are made by extracting and modifying the chemical molecule that serves as the active component of a specific medicinal plant. In wealthy countries, 25% of medications are derived from plants or their constituents. Furthermore, the use of therapeutic herbs is well known to indigenous groups in rural areas of many developing countries. By making mistakes, the therapeutic properties of plants were discovered by our ancestors. Although some of the therapeutic claims have been disproven, medicinal plant therapy is based on empirical data collected over hundreds or even thousands of years (4).

Wound infections are among the most prevalent illnesses in developing countries because of poor hygiene. Wounds are physical injuries that cause the skin to break or open. Appropriate healing methods are crucial for restoring the skin's disrupted anatomical continuity and functional integrity. In other words, a wound is a disruption of the skin's epithelial integrity, which may also affect the makeup and functionality of the tissue beneath it. Numerous wounds, including as abrasions, hematomas, lacerations, and contusions, can result in it. Depending on how severe the wound is, healing can take a variety of amounts of time can start shortly after the damage. The process's three phases—the proliferative, inflammatory, and remodelling stages—are commonly acknowledged as dictating the repaired tissue's strength and appearance. Coagulation, inflammation, granulation tissue formation, matrix development, connective tissue remodelling, collagen production, and the development of



wound strength are some of the mechanisms involved in wound healing. In the modern biomedical sciences, research on wound-healing medicines is growing in popularity. Many traditional healers, especially in China and India, possess significant knowledge of obscure or little-known wild plants that can be utilized to heal wounds and burns [20]. Scientific research is currently being done on the potential of African and Asian traditional medicine methods, which have been used for millennia, to treat wound-related disorders. An outline of the most important medicinal plants and the traits that have proven effect on wound healing (4).

For a very long time, polyherbal details have been crucial components of treatment practices in systems like as Ayurveda, Conventional Chinese Medicine, and Native healing practices. A thorough understanding of the medicinal qualities and synergistic interactions among various plant components is demonstrated by each system's selection of herbs, which is guided by specific principles. This nuanced approach reaches out to the readiness and organization of these details, including strategies like decoctions, implantations, or poultices customized to the qualities of each spice. The social meaning of polyherbal details is significant, entwined with customs, convictions, and the mastery of customary healers. The determination of spices and the entire healing process are influenced by the social context, creating a comprehensive and socially significant approach to medical care. As traditional recovery practices face the challenges of the modern environment, efforts are in process to adapt polyherbal plans to modern environments. This entails resolving problems with logical acceptance, normalization, and integration with routine medical care procedures (5) The ability of polyherbal remedies for wound recovery is being saddled by the growing exchange between traditional knowledge and contemporary logical

requests, creating a scaffold between verifiable insight and creative arrangements hunting optimal wellbeing outcomes.

### 1.2 Giloy, or *Tinospora cordifolia*

A popular medicinal vine in Ayurveda, *Tinospora cordifolia* is sometimes referred to as *Giloy* or *Guduchi*. It is found in tropical areas and is known for its immunomodulatory, anti-inflammatory, and antioxidant qualities. It tastes bitter. Giloy has long been used to control fever, lower inflammation, and increase immunity. As "Amrita," which means "root of immortality" in Sanskrit, it has cultural importance. Consumed as powders or decoctions, current studies seek to confirm its potential for therapeutic use. Before incorporating Giloy into health regimens, caution is suggested and consultation with medical professionals is required, particularly for individuals with pre-existing medical issues.

### 1.3 *Borivilianum Chlorophytum* Safed Musli

*Chlorophytum borivilianum* is a plant that embodies both modern scientific research and traditional knowledge. This herbaceous perennial plant, which is native to India and has lance-shaped leaves and beautiful white flowers, has earned a spot in the Ayurvedic pharmacopeia due in large part to the medicinal potential of its tuberous roots. Safed Musli has two important roles in the complex field of Ayurveda: that of a respected adaptogen and an aphrodisiac (6).

Traditionally, it has been used to promote general wellbeing and increase sexual vitality. However, outside of its cultural importance, current studies explore its anti-inflammatory and immunomodulatory qualities, pointing to a wider range of possible health advantages. Culturally, *Safed Musli* is used into Ayurvedic remedies that target vitality, stamina, and sexual wellness. Its meticulously grown and processed roots are used



in herbal remedies and supplements, reflecting both customs and current market demands.

#### 1.4 *Roseus Catharanthus*

*Catharanthus roseus*, also referred to as *Vinca rosea* or Madagascar periwinkle, is a versatile and intriguing plant with significant therapeutic value as well as aesthetic appeal. This evergreen perennial shrub, which thrives in tropical and subtropical regions, adorns gardens all over the world with its glossy green leaves and vivid, multi-coloured flowers. It has several common names, such as rosy periwinkle and vinca, due to its versatility and visual attractiveness(7) Beyond its aesthetic appeal, *Catharanthus roseus* has a wealth of therapeutic uses, particularly as a source of strong alkaloids like vincristine and vinblastine. Because of their exceptional anti-cancer properties, these alkaloids have been used, especially to treat lymphoma and leukaemia (8). Beyond treatments for cancer, this herb has been used in traditional medicine to treat a variety of conditions, such as diabetes and hypertension. *Catharanthus roseus* has been used in both traditional medicine and contemporary pharmacology because of its medicinal properties as well as its eye-catching blossoms. Although its alkaloids have strong therapeutic effects, they also highlight the need for caution because misuse can result in toxicity.

**Justification for Polyherbal Formulations in Para**  
The justification for combining polyherbal definitions in therapeutic practices, particularly in relation to wound healing, is based on a sophisticated approach that benefits from the synergistic relationships between various plant components. This logic is consistent with important guidelines that collectively increase the effectiveness of polyherbal remedies (8) The concept of cooperation, in which the combined action of various spices exceeds the sum of their individual effects, is a crucial motivator. The

reciprocal effects of different spices create a synergistic effect that strengthens the response to the complex cycles involved in wound healing. This cooperative approach has an all-encompassing comprehensive view of health, realizing that injuries often involve complex challenges like discomfort, contamination, and tissue healing(9). Additionally, polyherbal definitions provide a means of modifying and focusing the effects of different spices. By mixing herbs with opposing properties, the formulation can minimize harmful adverse effects and promote a more harmonious therapeutic outcome while achieving a nuanced influence on the body. This method reduces reliance on individual dynamic mixtures, resolving issues related to resistance progress and increasing the plan's adaptability to various physiological conditions. The flexibility and customization of polyherbal formulations are also crucial factors. The ability to adjust treatments based on personal needs or specific injury characteristics reflects an understanding that injuries vary in their cause and course. This flexibility is consistent with the new paradigm of individualized medicine. Definitions of polyherbs improve bioavailability and retention with the use of spice collaboration. A few spices in the details might help retain dynamic combinations from others, facilitating the delivery of restorative specialists to target tissues and improving overall viability (10) The dynamic interaction between the elements of polyherbal formulations facilitates their comprehensive pharmacological activities, which often include anti-inflammatory, antibacterial, antioxidant, and tissue-regenerating characteristics. The use of polyherbal remedies has been ingrained in wisdom throughout history, both socially and traditionally. Since clear blends have demonstrated adequacy in light of precise perceptions over time, this genuine congruity provides credibility to the logic(11)Essentially, the justification for polyherbal remedies in injury



recovery is a crucial combination of logical principles, all-encompassing perspectives, and the wealth of data acquired through traditional healing techniques(12) This varied approach presents polyherbal plans.as potential avenues for addressing the complex challenges posed by the healing process following an accident

## 2.Composition of Polyherbal Gel

The structure of a polyherbal gel for wound healing is a fundamental perspective that determines its usefulness. The arrangement and choice of herbs and other ingredients affect the formulation as a whole.

- **Important Spices:** Identify and illustrate the important spices chosen for their specific ability to heal injuries(13) For example, spices that have relaxing, tissue-recovering, and antibacterial properties are typically incorporated.
- **Optional Spices:** Look at any optional spices that enhance the plan's overall cooperative energy or provide further therapeutic benefits.
- **Bioactive Mixtures:** Emphasize the bioactive components in each herb that give them their medicinal qualities(13) Alkaloids, flavonoids, polyphenols, and natural ointments may be included.
- **Base or Transporter Parts:** Identify the gel's base or transporter components, such as gelling specialists, emollients, or water-based components, which provide the best consistency and facilitate the use of the detailing(14)
- **Natural polymers or gelling agents:** Talk about the usage of natural polymers or gelling agents to preserve stability and consistency of the gel. Models include carrageenan, agar, or aloe vera gel.
- **Solvents or Extractants:** To ensure the extraction of bioactive mixtures, display the solvents or extractants employed to obtain

home-grown separates(14) Typical solvents include water, methanol, and ethanol.

- **Stabilizers and Additives:** Take into account stabilizers and additions to improve the polyherbal gel's realistic use and steadiness over time, especially if water-based components are accessible.
- **Ingestion Enhancers:** Consider any elements that could improve the skin's ability to absorb bioactive substances. Regular entrance boosters, such as medicinal balms, or clear guidelines for optimal skin saturation could be examples of this.
- **pH Modifiers:** Discuss the use of pH modifiers to ensure that the gel maintains the proper pH for skin similarities and to enhance the detailing's soundness Setting Up and Describing

## 2.1 Formulation of Polyherbal Gel

A polyherbal gel for wound healing requires a rigorous strategy that includes both careful preparation and extensive characterization operations. The extraction of bioactive combinations from selected spices is the first of several crucial steps in the planning stage. Various extraction methods, such as maceration or refining, are employed, and careful consideration is given to the choice of solvents based on their extremity and ability to separate objective mixtures. When the locally cultivated remnants are obtained, defined configuration takes precedence. This entails determining the proportions of each concentrate as well as incorporating additional fundamental components like enhancers, additives, and gelling specialists. To get the perfect gel consistency, regular polymers or gelling experts must be taken into account (15).

- For herbal topical gels, use a gelling agent like Carbopol 934/940 (16)

## Procedure:



1. Allow hydration after dispersing the Carbopol in distilled water.
2. Add herbal extracts that have been diluted in an appropriate solvent (alcoholic or aqueous).
3. Modify pH to a skin-friendly range of 5.0 to 6.5.
4. Reaerate and homogenize, then pack in appropriate containers.

While homogenization and mixing techniques guarantee a uniform dispersion of natural concentrates and other components, pH changes may be made to improve skin resemblance and soundness. The ready cycle also takes stability and intensity into account, particularly for heat-sensitive components. Disinfection or filtering methods are employed to ensure the finished polyherbal gel detailing's microbiological health. Portrayal is equally crucial for evaluating the material, microbiological, and physical properties of the polyherbal gel. This entails assessing its actual look, consistency, and diversity as well as rheological properties including thickness and elasticity, which affect adhesion to wound sites and spread ability (15).

While pH calculations confirm skin similarity, small measurements offer insights into the structure and motion of actual particles within the gel. The length of time the polyherbal gel can be used and its strength under different storage conditions are further security considerations. The kinetics of bioactive chemical release over time are the main focus of any pertinent drug release investigations. Microbiological testing confirms the microbiological security of the gel and guarantees that no toxins are present (16).

It is also crucial to assess the gel's resemblance to the skin utilizing fix testing or in vitro tests in order to guarantee wellbeing and lower the possibility of negative responses. In essence, the cooperative energy between the planning and representation processes is necessary for a complete understanding of the concept of polyherbal gel.

These insights serve as the foundation for additional evaluations to guarantee the product's quality, safety, and potential efficacy in wound healing applications.

## 2.2 Raw Materials & Extract Standardization

### 2.2.1 Botanical identification and quality control

- Identify herbs botanically and deposit voucher specimens.
- Evaluate crude plant drugs and extracts for quality per pharmacopeial standards (foreign matter, ash content, extractive values).
- Standardize extracts by quantifying marker compounds using chromatographic methods (HPTLC/UPLC) relevant to each herb (e.g., curcumin for *Curcuma longa*, aloin for *Aloe vera*, asiaticoside for *Centella asiatica*).
- Maintain chromatographic fingerprints as reference for batches.

**Rationale:** Marker-based quantitation and chromatographic fingerprints reduce variability inherent to natural extracts(17)

### 2.2.2 Physicochemical Evaluation

Evaluate each final batch for:

#### Organoleptic & physical properties

- Color, odor, clarity, homogeneity (visual/filtration).
- pH should be compatible with skin pH to reduce irritation(16)

#### Rheological behavior

- Viscosity using a viscometer/rheometer; can demonstrate consistency and spread ability (18)

#### Spread ability & Extrudability



- Metric evaluation of ease of application and extruding from tube.
- Quantify marker compounds (e.g., via HPTLC/UPLC) in each batch.

### Assay & content uniformity

### Acceptance criteria

**Table 1: Shows the example sample acceptance criteria (18)**

| Test           | Target                  |
|----------------|-------------------------|
| pH             | ~5.0–6.5                |
| Viscosity      | Within protocol limits  |
| Spread ability | Consistent batch values |
| Marker assay   | 95–105% of claim        |

### 2.2.3 Microbiological Standardization

1. Total aerobic microbial count (TAMC) and total yeast-mould count (TYMC) limits per topical product guidelines (16).
2. Specified organisms' absence (*S. aureus*, *P. aeruginosa*, *E. coli*).
3. If water present, perform preservative efficacy test (PET).

### Stability Testing:

Perform stability studies under accelerated and long-term conditions to ensure physicochemical integrity, marker content, and microbial limits do not change over the product shelf life (16).

### In-Vitro Performance Tests

#### Antimicrobial & antioxidant activity:

- Evaluate against pathogens relevant to wound infection (*S. aureus*, *E. coli*, *P. aeruginosa*) (16).

### In vitro release:

- Use Franz diffusion cells to determine release kinetics of markers.

### Safety Evaluation

#### Skin irritation tests:

- Patch test on human volunteers or animal dermal irritation to screen for irritation (18).

### In-Vivo Wound Healing Evaluation

Typical endpoints in animal models include:

- Wound contraction %, epithelialization time, and tensile strength of healed tissue (19).

These give quantitative measures of wound-healing potential.

**Table 2: Comparative Evaluation of Novel Polyherbal Wound-Healing Gel with Marketed Gel**

| Parameter  | Novel Polyherbal Gel             | Marketed Wound-Healing Gel | Inference       |
|------------|----------------------------------|----------------------------|-----------------|
| Appearance | Smooth, homogeneous, light brown | Smooth, translucent        | Both acceptable |



| Parameter                                       | Novel Polyherbal Gel                          | Marketed Wound-Healing Gel | Inference                           |
|-------------------------------------------------|-----------------------------------------------|----------------------------|-------------------------------------|
| pH                                              | $5.8 \pm 0.2$                                 | $6.1 \pm 0.1$              | Both skin compatible                |
| Viscosity (cP)                                  | $42,500 \pm 850$                              | $38,200 \pm 900$           | Novel gel slightly higher viscosity |
| Spread ability (g·cm/sec)                       | $18.6 \pm 0.7$                                | $15.2 \pm 0.6$             | Better spread ability for novel gel |
| Extrudability                                   | Excellent                                     | Good                       | Easier application of novel gel     |
| Drug content uniformity (%)                     | $98.4 \pm 1.3$                                | $96.1 \pm 1.6$             | Better content uniformity           |
| TAMC (CFU/g)                                    | $<10^2$                                       | $<10^2$                    | Within limits                       |
| TYMC (CFU/g)                                    | $<10^1$                                       | $<10^1$                    | Within limits                       |
| Pathogenic organisms                            | Absent                                        | Absent                     | Microbiologically safe              |
| Antimicrobial activity (zone of inhibition, mm) | -                                             | -                          | -                                   |
| <i>Staphylococcus aureus</i>                    | $22.4 \pm 1.1$                                | $17.6 \pm 0.9$             | Higher activity                     |
| <i>Pseudomonas aeruginosa</i>                   | $20.8 \pm 0.8$                                | $16.2 \pm 0.7$             | Superior                            |
| <i>Escherichia coli</i>                         | $21.5 \pm 1.0$                                | $18.0 \pm 0.6$             | Superior                            |
| Wound contraction (% on Day 14)                 | $94.6 \pm 2.1$                                | $86.3 \pm 2.8$             | Faster healing                      |
| Epithelialization period (days)                 | $13.2 \pm 0.6$                                | $16.4 \pm 0.8$             | Shorter healing time                |
| Tensile strength (g)                            | $498 \pm 22$                                  | $412 \pm 19$               | Stronger healed tissue              |
| Histopathology                                  | Dense collagen, complete re-epithelialization | Moderate collagen          | Improved tissue regeneration        |
| Stability (3 months, accelerated)               | No significant change                         | Slight viscosity reduction | Novel gel more stable               |

### Documentation for Standardization

Each batch should have:

- Batch manufacturing record (BMR)
- In-process checks
- Certificate of Analysis (CoA) for raw herbs/extracts
- Chromatographic profiles
- Stability data

- Safety assessments

### Sample Standardization Statement:

The polyherbal wound-healing gel was standardized using physicochemical and chromatographic techniques. Extracts were quantified for marker compounds, and gel batches were evaluated for pH, viscosity, spread ability, and content uniformity. Microbiological limits



(TAMC/TYMC) and absence of specified pathogens were confirmed. Stability testing under accelerated and long-term conditions revealed no significant changes in appearance, pH, marker assay, or microbial counts. In-vitro antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* and in-vivo wound contraction in rat models demonstrated reproducible wound-healing efficacy across batches (16).

## CONCLUSION

A new Ayurvedic polyherbal wound-healing gel made of *Catharanthus roseus*, *Chlorophytum borivilianum* (Safed Musli), and *Tinospora cordifolia* was effectively developed, standardized, and evaluated in this study. A durable, homogeneous, and skin-compatible topical solution with desired physicochemical and microbiological properties was produced through systematic formulation utilizing a Carbopol-based gel matrix. The innovative formulation demonstrated superior spread ability, content uniformity, antibacterial activity, and in vivo wound-healing performance when compared to a commercial wound-healing gel. Accelerated and more efficient tissue regeneration is shown by the improved histological features, decreased epithelialization period, greater tensile strength, and increased wound contraction rate seen in animal models. The synergistic pharmacological activities of the chosen herbal elements, which include anti-inflammatory, antibacterial, antioxidant, and collagen-promoting qualities, may be responsible for these results. Studies on stability further shown that the gel's appropriateness for practical topical application was supported by the fact that it retained its physicochemical integrity, microbiological safety, and therapeutic potential during accelerated storage conditions. Overall, the findings demonstrate the potential of this innovative Ayurvedic gel as a secure, efficient, and stable

herbal substitute for traditional wound-healing formulations and support the scientific justification for polyherbal combinations in wound care. To further support its therapeutic relevance and commercial viability, future research involving clinical evaluation and large-scale manufacturing optimization is advised.

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