2025 Volume: 01 Issue: 04 www.ijrpa.com Research Article

International Journal Research Publication Analysis

Page: 87-110

FORMULATION AND EVALUATION OF A MULTICOMPONENT TOPICAL OINTMENT FOR WOUND HEALING PROPERTIES

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Article Received: 08 July 2025
Article Revised: 28 July 2025
Published on: 18 August 2025

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ABSTRACT

Wound healing, often hindered by infection and inflammation, typically requires complex, multi-product treatments. This study developed and evaluated a novel, multi-component topical ointment designed for comprehensive wound care. The formulation incorporates sulfanilamide, copper sulfate, zinc oxide, and boric acid for their combined antimicrobial, anti-inflammatory, astringent, and tissue-regenerating properties. Four ointment variations (F1-F4) were prepared using a petrolatum-based vehicle and subjected to rigorous physicochemical and biological assessments. All formulations exhibited favorable organoleptic qualities, skin-compatible pH (5.4-6.5), and optimal spreadability and viscosity, indicating good user acceptance and application consistency. Importantly, skin irritation tests confirmed their dermal safety, showing no signs of irritation. In vitro studies demonstrated significant efficacy: the ointment exhibited broad-spectrum antimicrobial activity against *Staphylococcus aureus* (7mm zone of inhibition) and *Candida albicans* (6mm zone). Drug diffusion studies confirmed sustained release, with 85.10% cumulative release over 90 minutes. Furthermore, a cytotoxicity assay using L929 fibroblast cells revealed the ointment's pro-healing potential, achieving complete wound closure within 48 hours, comparable to a standard positive control. Among the tested formulations, Formulation 1 (F1) consistently demonstrated superior physicochemical characteristics, optimal drug diffusion, potent antimicrobial effects, and excellent cell viability. This research successfully delivered a stable, safe, and effective multi-functional topical ointment, offering a streamlined,

comprehensive solution for wound management, especially valuable in resource-constrained environments.

KEYWORDS: Wound healing, Topical ointment, Multifunctional, Sulfanilamide, Copper sulfate, Zinc oxide, Boric acid, Antimicrobial, Anti-inflammatory, Tissue regeneration, Physicochemical evaluation, Spreadability, Viscosity, pH, Skin irritation, Drug diffusion, Cytotoxicity, L929 cell line.

INTRODUCTION

Wound healing is an essential and highly regulated biological process that restores skin and tissue integrity following injury. It is broadly classified into four overlapping but distinct phases: hemostasis, inflammation, proliferation, and remodeling. Successful wound healing requires coordinated cellular and molecular responses across these stages, including clot formation, immune cell recruitment, new tissue synthesis, and extracellular matrix remodeling. Disruption in any of these stages—especially inflammation—can lead to delayed healing, chronic wounds, or infection, particularly in patients with comorbidities like diabetes, vascular disorders, or immunodeficiency.

Phases of Wound Healing

1. **Hemostasis Phase**

- Occurs immediately after tissue injury.
- Platelets aggregate at the wound site to form a clot via the coagulation cascade.
- Fibrin mesh forms a provisional matrix that stabilizes the wound and acts as a scaffold for incoming cells.

2. **Inflammation Phase**

- Lasts for hours to days.
- Characterized by vasodilation and increased vascular permeability mediated by histamine and cytokines.
- Neutrophils and macrophages migrate to the site to clear pathogens and debris.
- Macrophages release cytokines and growth factors that facilitate the transition to the proliferative phase.

3. **Proliferation Phase**

- Involves angiogenesis, fibroblast proliferation, extracellular matrix deposition, and epithelialization.

- Granulation tissue forms, comprising collagen, fibronectin, hyaluronic acid, and new capillaries.
- Myofibroblasts mediate wound contraction.
- Keratinocytes migrate across the wound bed to re-establish the epidermal barrier.

4. Remodeling Phase

- Can last from weeks to years depending on wound severity.
- Collagen type III is replaced by type I to increase tensile strength.
- The vascularity of the wound diminishes as remodeling progresses.
- Final scar formation occurs, although the strength of the tissue never returns to that of uninjured skin.

Fig. 1: Accidental wound of a patient.

Challenges in Modern Wound Care

Despite therapeutic advancements, wound care remains challenging, particularly for chronic or infected wounds. These wounds often remain in the inflammatory phase, have impaired blood flow, and may harbor biofilms that resist treatment. Thus, wound care demands products that not only treat infection but also support healing holistically.

Role of Multi-Functional Topical Agents

An ideal wound care product should offer multiple therapeutic effects:

- **Antimicrobial Action:** To control infection, especially biofilms in chronic wounds.
- **Anti-inflammatory Effects:** To modulate, not suppress, inflammation.
- **Promotion of Tissue Repair:** Stimulate fibroblast activity, angiogenesis, and epithelialization.
- **Moisture Retention:** A moist wound environment enhances healing.
- **Biocompatibility and Biodegradability:** Non-irritating, non-toxic formulations.
- **Sustained Drug Release:** Maintain therapeutic drug levels at the wound site.

Advantages of Topical Dosage Forms

Topical delivery systems provide several advantages in wound care:

1. **Localized Action:** High drug concentrations at the site with minimal systemic absorption.
2. **Avoidance of First-Pass Metabolism:** Direct application bypasses hepatic metabolism.
3. **Non-Invasive and Patient-Friendly:** Suitable for home or outpatient settings.
4. **Potential for Sustained Release:** Formulations can be tailored to release drugs over time.
5. **Direct Contact with Wound Tissue:** Allows for targeted delivery and optimal pharmacodynamic response.

Ointments in Wound Management

Among topical forms, **ointments** are especially beneficial due to their occlusive nature:

- **Occlusivity and Moisture Retention:** Maintain a moist environment, reducing TEWL.
- **Enhanced API Penetration:** Occlusion hydrates the stratum corneum, increasing permeability.
- **Barrier Protection:** Shields against contaminants and mechanical irritation.
- **Formulation Versatility:** Can be customized with multiple APIs for synergistic effects.

Ointment Bases: Types and Relevance

1. **Oleaginous Bases:** Highly occlusive, ideal for dry wounds. (e.g., petrolatum)
2. **Absorption Bases:** Absorb water to form W/O emulsions, moderate occlusion.
3. **Water-Removable Bases (Emulsions):** Non-greasy, good for exudating wounds.
4. **Water-Soluble Bases:** PEG-based, non-occlusive, ideal for moist/infected wounds.

Rationale for Combinational Topical Therapy

Wound healing is multi-phasic; single-agent formulations often fall short. Combining multiple APIs can:

- **Provide Synergistic Effects:** Enhance antimicrobial, anti-inflammatory, and regenerative actions.

- **Broaden Spectrum of Activity:** Effective against polymicrobial infections.
- **Reduce Resistance Risk:** Combination reduces potential for microbial resistance.
- **Simplify Regimens:** Improves patient compliance and treatment efficacy.
- **Cost Efficiency:** Reduces need for multiple products, especially in rural settings.

Significance of the Study

This study aims to develop a **combinational ointment** containing sulfanilamide, copper sulfate, zinc oxide, and boric acid—each with complementary therapeutic actions. The formulation seeks to:

- Deliver broad-spectrum antimicrobial protection.
- Offer anti-inflammatory and astringent properties.
- Promote angiogenesis and tissue regeneration.
- Be stable, cost-effective, and suitable for wide-scale use.

Identified Research Gaps

1. **Lack of Comprehensive Products:** Existing products often target single aspects (e.g., infection) but neglect others like inflammation or regeneration.
2. **Underutilization of Known Drugs:** Many older drugs with proven efficacy are not explored in modern combinations.
3. **Accessibility Issues:** High-end therapies are unaffordable or unavailable in resource-limited settings.

Addressing the Gap

This study bridges these gaps by:

- Rationally combining **multiple proven APIs** in a single ointment base.
- Ensuring **physicochemical compatibility** and **synergistic activity**.
- Developing a **clinically relevant**, **economical**, and **multi-functional** product.

Such innovation aligns with the growing need for efficient, accessible, and patient-friendly wound therapies that support the healing process holistically from infection control to tissue remodeling.

MATERIALS

Chemicals Required

Table 1: Chemicals Required.

Ingrident	Uses
Boric acid	Antiseptic, antifungal
Zinc oxide	Antimicrobial
Sulfanilamide	Antibacterial
Copper sulphate	Antifungal
Glycerine	Humectant, moisturizer
Beeswax	Thickening agent
Benzyl alcohol	Preservative
Methyl paraben	Preservative
Petroleum jelly	Ointment base
Lanolin	Ointment base

Materials Required

1. Mortar and pestle
2. Water bath
3. Beaker
4. Stirrer
5. Thermometer
6. Sterile container
7. Weighing balance
8. Spatula
9. 80 mm sieve
10. Digital ph meter
11. Brookfield viscometer
12. Glass slides
13. Cellophane membrane
14. L929 cell line

Micro Organisms Required

1. Staphylococcus aureus

2. Candida albicans

Formulation Development

In the present study, four different topical formulations, labeled as F1, F2, F3, and F4, were developed by varying the concentrations of the active pharmaceutical ingredients (APIs). The

formulation strategy focused on achieving optimal spreadability, stability, and therapeutic effectiveness for wound healing. The preparation process involved the following key steps:

1. **Weighing and Pre-blending of APIs**

✧ Accurately weighed quantities of active pharmaceutical ingredients were measured using a digital analytical balance to ensure dose accuracy.

✧ The APIs were selected for their complementary roles in antimicrobial activity, astringency, and epithelial regeneration.

✧ Each ingredient was sieved through an 80-mesh sieve to ensure uniform particle size, reduce clumping, and enhance blendability.

✧ The APIs were initially triturated using a mortar and pestle to achieve a uniform powder mixture and minimize particle aggregation.

2. **Preparation of the Base**

✧ A skin-compatible, semi-solid base was prepared using a blend of emollients and stabilizers to enhance product consistency and patient comfort.

✧ The base composition included:

➢ Petroleum Jelly – for occlusion and hydration.

➢ Beeswax – for structural integrity and spreadability.

➢ Glycerin – as a humectant to draw moisture and maintain a moist wound environment.

✧ These ingredients were melted together using a double-boiler method in a water bath, avoiding direct heat to preserve the thermal stability of the excipients.

3. **Incorporation of APIs into the Base**

✧ The previously blended API mixture was gradually incorporated into the molten base using geometric dilution, ensuring uniform dispersion throughout the formulation.

✧ Continuous stirring was performed using a glass rod or magnetic stirrer, maintaining homogeneous mixing at a temperature range of 60–70°C, depending on the melting points of the components.

✧ The mixing was carried out on an ointment slab to facilitate manual homogenization and achieve the desired semi-solid consistency.

4. **Homogenization**

✧ The formulations were subjected to gentle stirring or mechanical homogenization during the cooling phase to ensure uniform texture and prevent phase separation.

✧ The mixture was allowed to cool gradually at room temperature, with occasional stirring

to avoid air entrapment and ensure a smooth finish.

5. Filling and Storage

- ✧ Once cooled to a semi-solid consistency, the formulations were transferred into pre-sterilized, airtight aluminium or plastic containers using sterile spatulas. Containers used were light-resistant (amber-colored) to protect light-sensitive ingredients from degradation.
- ✧ Each batch was properly labeled with the formulation code (F1–F4), date of preparation, and storage conditions.
- ✧ All samples were stored at room temperature (25 ± 2°C) in a clean, dry environment away from direct sunlight and moisture.

Fig. 2: Formulated Oinnment.

Formulation: 1

Table 2: Formulation 1 Ingredients.

Ingredients	Qty(g)	Properties
Boric Acid	2	Antiseptic, Antifungal
Zinc Oxide	10	Antiinflamatory
Copper Sulphate	0.5	Antimicrobial
Sulfanilamide	2	Antibacterial
Petroleum Jelly	q.s	Base

Fig. 3: Formulation 1.

Formulation 2

Table 3: Formulation 2 Ingredients.

Ingredients	Qty(g)	Properties
Boric Acid	2	Antiseptic & Antifungal
Zinc Oxide	10	Antiinflamatory
Sulfanilamide	2	Antibacterial
Copper Sulphate	0.5	Antimicrobial
Glycerine	13ml	Humectant & emollient
Petroleum Jelly	q.s	Base

Fig. 4: Formulation 2.

Formulation 3

Table 4: Formulation 3 Ingredients.

Ingredients	Qty(g)	Properties
Zinc oxide	15	Antiinflamatory
Boric Acid	5	Antiseptic, Antifungal
Sulfanilamide	4	Antibacterial
Copper Sulphate	1	Antimicrobial
Glycerine	5.5ml	Humectant & emollient
Beeswax	12	Healing, Moisturizers
Methyl Paraben	0.3	Preservative
Petroleum Jelly	56	Base

Fig. 5: Formulation 3.

Formulation 4

Table 5: Formulation 4 Ingredients.

Ingredients	Qty(g)	Properties
Boric Acid	2	Antiseptic, Antifungal
Zinc oxide	10	Antiinflamatory
Sulfanilamide	2	Antibacterial
Copper Sulphate	0.5	Antimicrobial
Glycerine	3	Humectant & emollient
Beeswax	10	Healing, Moisturizers
Benzyl Alcohol	0.5	Preservative
Petroleum Jelly	67	Base

Fig. 6: Formulation 4.

Evaluation parameters for the formulated wound healing ointments

1. Organoleptic evaluation

Purpose

To assess the visual and sensory qualities of the ointment that influence user acceptance and formulation stability.

Parameters Assessed

Color: Should be uniform, matching the formulation design.

Odor: Should be pleasant or neutral, indicating the absence of degradation or microbial growth.

Texture: Smooth, non-gritty, and easily spreadable.

Appearance: Semi-solid, homogeneous, and free from lumps, crystals, or phase separation.

Acceptable Criteria

Color: Uniform, no discoloration

Odor: Mild or odorless

Texture: Smooth, non-gritty

Stability: No separation or crystallization upon storage

Obtained Results

Color: Uniform greenish blue color

Odor: odorless

Texture: Smooth, non-gritty

Stability: No separation

2. PH Determination

Purpose

To ensure the ointment is compatible with skin pH (4.5–6.5) and does not cause irritation or discomfort.

Method

A 1% w/v dispersion of the ointment was prepared in distilled water.

pH was measured using a calibrated digital pH meter at room temperature.

Acceptable Range

4.5 to 6.5 – Suitable for topical application and skin tolerance.

Results

Batch no: 1 (6.0)

Batch no: 2 (6.5)

Batch no: 3 (5.8)

Batch no: 4 (5.4)

3. Spreadability Test

Purpose

To assess how easily the ointment spreads on the skin surface, affecting ease of application and patient comfort.

Method

A fixed amount of ointment was placed between two glass slides. A 500 g weight was applied to the upper slide.

Time (T) required for the upper slide to move a measured distance (L) was recorded using a stopwatch.

Formula

Where

S = Spreadability (g·cm/s)

M = Weight (g)

L = Distance moved (cm)

T = Time taken (s)

Spreadability (S)=M×L/T

Acceptable Range

The greater the S Value better the spreadability of the formulation

Results

Table 6: Spreadability Results.

Formulation Code	Length (L)	Mass required to slide (M)	Time taken for sliding(T)	Spreadability S= (LxM)/T
1	5.5	26.29	26	5.56
2	5.5	25.49	34	4.23
3	5.5	41.79	53	4.34
4	5.5	32.19	42	4.21

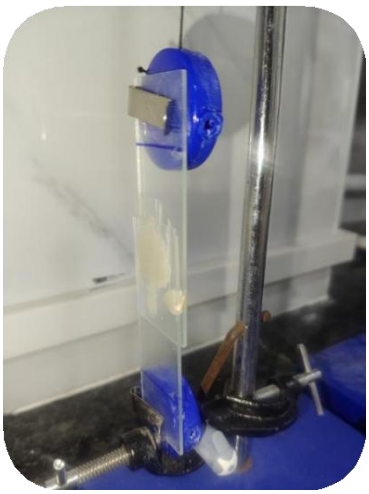

Fig. 7: Spreadability apparatus.

Viscosity

Purpose

To measure the resistance to flow and determine application consistency and stability.

Method

Viscosity was measured using a Brookfield viscometer (BV)at a fixed RPM and temperature (e.g., 25°C).

Spindle number and speed were chosen based on sample consistency (usually Spindle 64, 10–20 RPM).

Acceptable Range

5,000 – 100,000 cP (centipoise) depending on the type of ointment.

Topical ointments typically show moderate to high viscosity for better retention on skin.

Viscosity Report

NAME OF THE SAMPLE : OINTMENT – SEMISOLID

DATE OF ANALYSIS : 02-05-2025

TYPE OF VISCOMETER : DV2T BROOKFIELD VISCOMETER TYPE ODF

SPINDLE : T2

SHEAR RATE : 0.5

TEMPERATURE : 34.3 ^{o}C RESULTS

Table 7: Viscosity Results.

Sample	Viscosity
Sample 1	90000 cP
Sample 2	100500 cP
Sample 3	97840 cP
Sample 4	98020 cP

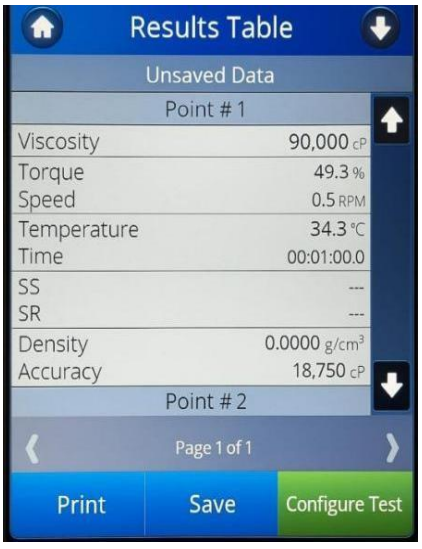

Fig. 8: Picture of Viscometer Results.

4. Skin Irritation Test

Purpose

To evaluate **dermal compatibility and safety** of the formulation.

Method

Applied 1 g of ointment on the shaved dorsal surface of healthy albino rats or rabbits (or human volunteers for in vivo patch test, if approved).

Observed for 24–72 hours for signs of redness, itching, edema, or other reactions.

Scoring Criteria

0 = No reaction

1 = Slight erythema

2 = Moderate erythema

3 = Severe erythema with edema

Acceptable Result

No irritation or mild reaction (score 0–1)

Results

No irritation (Score 0)

5. Antimicrobial Activity (Including Bacteria And Fungi)

Purpose

To evaluate the ointment's broad-spectrum antimicrobial efficacy against common wound pathogens.

Methodology

The anti-microbial properties of the ointment were assessed using the agar well diffusion technique, as described by Holder and Boyce (1994). The bacterial and fungal strains employed in this study included Gram-positive *Staphylococcus aureus*, and *Candida albicans*. The test organisms were initially cultured in nutrient broth for bacteria and Potato dextrose broth for fungi and incubated at 37 °C for 24 hours. Subsequently, an overnight culture was uniformly spread over Mueller-Hinton agar plates using a sterile cotton swab to ensure even microbial distribution. Wells measuring 6 mm in diameter were made in the agar using a sterile cork borer.

For controls, Chloramphenicol (1 mg/mL) was used as a positive control for Gram-positive strains, Fluconazole (1 mg/mL) for *Candida albicans*, and DMSO alone served as the negative control. Each well was loaded with the respective test sample or control solution. The plates were then incubated at 37 °C for 24 hours. After incubation, the zones of inhibition around the wells were measured in millimetres to determine the anti-microbial effectiveness of the ointment by comparing them with the control treatments.

RESULTS

The agar well diffusion technique evaluates the anti-microbial potential of ointment by measuring the diameter of the clear zones (zones of inhibition) surrounding wells which reflect the degree of microbial growth suppression.

In this experiment, the anti-microbial effects of ointment were examined using *Staphylococcus aureus* and *Candida albicans*, which were cultured on Mueller-Hinton Agar (MHA) plates. The outcomes are presented in Table 1 and Figure 1, while Table 2 illustrates the percentage inhibition of growth. The observed inhibition zones highlight the most effective anti-bacterial action of the ointment formulation. Among the tested pathogens, *Staphylococcus aureus* showed the highest inhibition zone of 7 mm, followed by *Candida albicans* with a 6 mm zone of inhibition.

Table 8: Anti-microbial effect of ointment

Test pathogens	Zone of Inhibition (in mm)		
	Ointment	Positive Control	Negative Control
Staphylococcus aureus	7	12	No ZOI
Candida albicans	6	16	No ZOI

Table 9: Percentage of Anti-microbial effect of ointment.

Test Pathogens	Ointment
Staphylococcus aureus	58.33
Candida albicans	37.5

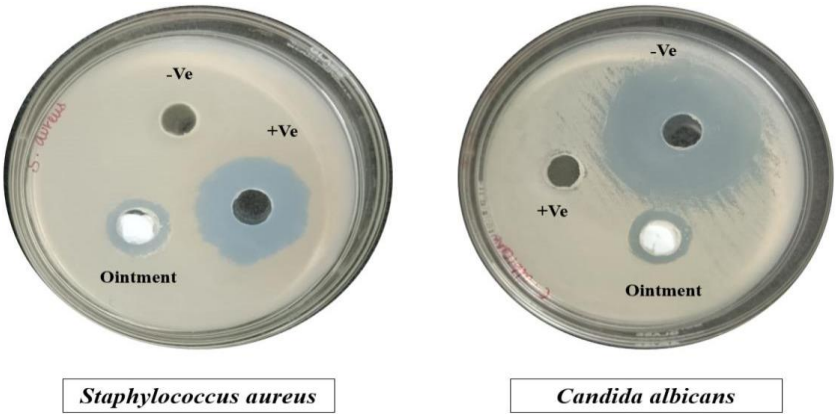

Fig. 9: Percentage of anti-bacterial growth inhibition

6. In Vitro Drug Diffusion Study (Franz Diffusion Cell)

Purpose

To determine the rate and extent of drug release from the ointment through a semi-permeable membrane.

Method

Franz diffusion cell with.

Cellophane membrane (pre-soaked) Donor compartment: 1 g ointment.

Receptor compartment: phosphate buffer (pH 7.4) at **37 ± 1°C**, stirred continuously Samples withdrawn at intervals (0.5, 1, 2, 4, 6, 8 hours), replaced with fresh buffer. Analyzed using UV spectrophotometry.

Acceptable Outcome

Sustained and controlled drug release over 6–8 hours

Cumulative % drug release ≥ 70% is generally considered effective.

Results

Table 10: Calculated Result of Diffusion Analysis.

S. No.	Time	Absorbance	y+c	Concentar ion (x) µg/ml (x=y-c/m)	Concentarion (x) mg/ml	Dilution (x20)	Concentration in 2ml	Concentration in 30 ml	Cumula tive amount release	% CDR
1	10	0.91	0.9126	9.7292	0.00972	0.194	0.389	5.8375	5.837	29.19
2	20	0.98	0.9826	10.475	0.0104	0.209	0.419	6.2852	6.674	33.37
3	30	1.4	1.4026	14.953	0.0149	0.299	0.598	8.9718	9.780	48.90
4	45	1.65	1.6526	17.618	0.0176	0.352	0.704	10.571	11.97	59.89
5	60	1.92	1.9226	20.496	0.0204	0.409	0.819	12.298	14.40	72.05
6	90	2.2	2.2026	23.481	0.0234	0.469	0.939	14.089	17.02	85.10

Fig. 10: France Diffution Apparatus.

7. Cytotoxicity Study Using L929 Cell Line

Purpose

To assess the biocompatibility and cytotoxicity of the ointment using mouse fibroblast L929 cells — a standard for wound healing models.

Methodology Chemicals and reagents

Dulbecco's Modified Eagle's medium (DMEM) (Himedia), Fetal Bovine Serum (Himedia), Delbucco's Phosphate Buffered Saline (DPBS) (Himedia), and Cipladine (Cipla Ltd).

Cell Culture

The L929 mouse fibroblast cell line was obtained from the National Centre for Cell Science (NCCS), Pune, and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics (100 U/mL each of streptomycin and penicillin). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and were subcultured upon reaching approximately 80% confluency. A hemocytometer was used for cell counting, and viability was evaluated to ensure accurate seeding densities for subsequent assays.

Scratch assay

The wound healing activity of the ointment was evaluated using an in vitro cell migration (scratch) assay on L929 cells, following a previously established protocol. Briefly, 10,000 cells per well were seeded into 6-well plates and incubated overnight to allow for attachment.

The following day, cells were washed with DPBS, and a linear scratch was created using a sterile 200 µL pipette tip. To remove dislodged cells and debris, the wells were rinsed again with DPBS. Cells were then treated with 1000 µg/mL of the test compound and 5 µg/mL of the positive control drug Cipladine, known for its wound healing properties (Liang et al., 2007; Kumar et al., 2007), and incubated for 24 hours. Cellular movement and morphological alterations were monitored using an inverted microscope equipped with a digital camera. The extent of wound closure and scratch width was quantified at 24, 36, and 48 hours using the ImageJ software.

Wound Closure Percentage Formula:

$$\text{Wound Closure } (\%) = \left(\frac{\text{Initial Width} - \text{Width at Time}}{\text{Initial Width}} \right) \times 100$$

RESULTS

L929 cells were grown in a 24-well plate at a density of 10,000 cells for each well. A 200 µL pipette held vertically to the orifice plate from the top down was employed to create scratches. The medium was removed, after adding the sample, images were captured at various time intervals such as 24hr, 36hr, and 48hr (Figure 1). The main steps in the wound healing process consist of fibroblasts being activated, multiplying, and moving, involving different cell types and additional micro environmental elements. The scratch assay is a frequently utilized method in vitro to investigate the wound healing abilities of medically important substances (Liang et al., 2007). In this research, L929 cells were subjected to a test sample for 48 hours. Cell migration was documented at 24, 36, 48 hours and the extent of wound closure was assessed with Image J software.

The results indicated that the test sample eliminated the scratch gap completely within 48 hours. Table 1 displays the percentage of wound closure at different time intervals in the control group (administered with the standard drug). The test sample displayed L929 cells to move, resulting in the wound's closure. Likewise, the cells exposed to the standard drug had completely closed the gap after 48 hours. The images show increased cell movement in cells treated with the control drug and cells treated with the test samples. Table 2 and Figure 2 displayed the percentage of wound closure in the experiment.

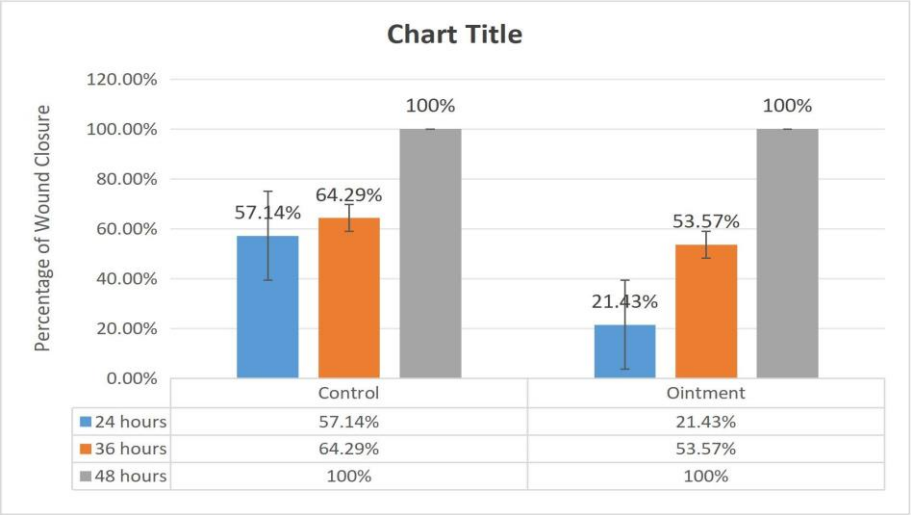

Fig. 11: Percentage of wound closure at different time intervals.

Table 11: Wound Area Measurement in Scratch Assay.

Sample Code	0 hours	24 hours	36 hours	48 hours
Control (Cipladine)	14 cm	8 cm	5 cm	0 cm
Ointment	14 cm	11 cm	6.5 cm	0 cm

Table 12: Percentage of Wound Area Closure in Scratch Assay.

Sample Code	24 hours	36 hours	48 hours
Control (Cipladine)	57.14%	64.29%	100%
Ointment	21.43%	53.57%	100%

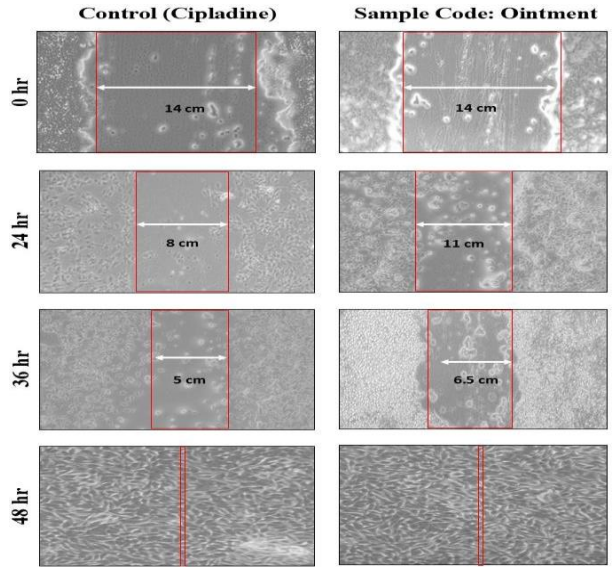

Fig. 12: Microscopic pictures illustrating the wound healing properties of a test sample (Ointment) in a controlled environment: L929 cells were exposed to the test sample and Cipladine drug, with images taken every 0, 24, 36, and 48 hours. The dark red line delineated the edges of the scratched wounds and to calculate the wound closure.

CONCLUSION

This research successfully formulated and characterized four different wound-healing ointments incorporating sulfanilamide, copper sulfate, zinc oxide, and boric acid in varied ratios. Among the formulations, Formulation 1 (F1) consistently exhibited superior performance in physicochemical properties, biological activity, and dermatological compatibility.

The enhanced diffusion profile of F1 ensures optimal drug release at the wound site, while its antimicrobial action provides a broad spectrum of protection against both bacterial and fungal infections. Additionally, its favorable pH, viscosity, and spreadability indicate patient-friendly handling and adherence to the affected area. Most notably, the cell line study confirmed the formulation's safety and potential to promote cell proliferation—an essential component in wound healing.

Therefore, Formulation 1 can be considered a promising candidate for further development as a topical wound-healing ointment. Future work should focus on long-term stability studies, in vivo wound healing assessments, and scale-up for commercial manufacturing to translate these findings into a clinically viable product.

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