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# Formulation, Antimicrobial, and Wound-healing Evaluation of a Polyherbal Gel Containing *Bixa orellana* L. and *Withania somnifera* (L.) Dunal

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## Abstract

**Background:** The escalating global burden of antimicrobial resistance has intensified the search for safe, plant-derived antimicrobial agents with therapeutic potential. **Aim:** To develop and evaluate a polyherbal topical gel formulated from methanolic leaf extracts of *Bixa orellana* L. and *Withania somnifera* (L.) Dunal for antimicrobial and wound-healing activity. **Methods:** Methanolic leaf extracts were prepared by Soxhlet extraction and screened phytochemically. Antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria, and a yeast was evaluated by the agar well diffusion method at 0.1 and 0.2 mg/ml. A polyherbal gel (2% and 5% w/w) was formulated using Carbopol-940 and evaluated for physicochemical properties, stability, and cutaneous safety. Wound-healing activity was assessed using an excision wound model in Wistar albino rats. **Results:** Extracts contained alkaloids, flavonoids, tannins, saponins, and terpenoids, with glycosides additionally detected in *W. somnifera*. The polyherbal combination showed enhanced antimicrobial activity at the lower test concentration, while *B. orellana* alone was comparable at the higher concentration; ofloxacin remained the most active agent overall. The gel showed acceptable pH, viscosity, spreadability, homogeneity, and stability under storage, with no signs of dermal irritation or acute toxicity. *In vivo*, the 5% gel produced wound contraction and epithelialization comparable to the standard drug. **Conclusion:** The polyherbal gel combining *B. orellana* and *W. somnifera* is a stable, safe, and effective topical candidate with antimicrobial and wound-healing potential, supporting its traditional use and further pharmaceutical development.

**Major Findings:** The 5% polyherbal gel matched standard-drug wound-healing efficacy in the excision wound model, and the extract combination showed a concentration-dependent, likely synergistic, antimicrobial effect that was most pronounced at the lower test concentration.

**Keywords:** Agar Well Diffusion, Antimicrobial Activity, *Bixa orellana* L., Carbopol-940, Methanolic Extract, Phytochemical Screening

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## 1. Introduction

The increasing global issue of Antimicrobial Resistance (AMR) is considered one of the significant hazards of the twenty-first century to public health. The rapid emergence of resistant bacterial strains, brought on by the widespread misuse and overuse of synthetic antimicrobial medications in clinical, veterinary, and agricultural settings, has rendered many conventional antibiotics ineffective. This has made previously

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controllable infections more difficult to manage, leading to longer hospital stays, higher mortality, and a substantial financial burden on healthcare systems worldwide. This concerning circumstance has raised the need for alternative antimicrobial approaches that are effective, safe, affordable, and sustainable.

Medicinal plants have been used to treat and prevent infectious diseases since ancient times. Long before contemporary antibiotics were developed, plant-based remedies were frequently used to treat microbial infections in traditional folk medicine, *Ayurveda* and *Unani* systems. Herbal remedies continue to provide basic medical care to a significant portion of the world's population. A variety of secondary metabolites present in plants, including flavonoids, tannins, alkaloids, terpenoids, saponins, phenolic compounds, and glycosides, have been shown to possess antibacterial properties.

The said-mentioned phytochemicals exhibit antimicrobial properties through a range of mechanisms, such as rupturing microbial cell membranes, inhibiting enzyme activity, interfering with nucleic acid synthesis, and decreasing microbial adherence and colonisation. The availability of multiple bioactive constituents allows plant extracts to act on multiple microbiological targets simultaneously, thus minimising the risk of resistance development compared with single-target synthetic antibiotics[1].

Among the wide array of medicinal plants, *B. orellana* L., commonly known as annatto, lipstick tree, or achiote, has gained considerable attention for its pharmacological significance. Traditionally used as a natural dye and medicinal agent, *B. orellana* L. has been reported to possess antimicrobial, anti-inflammatory, antioxidant, and antiseptic properties. The plant is particularly rich in carotenoid pigments such as bixin and norbixin, along with flavonoids, tannins, and terpenoids, which are known to contribute to its antimicrobial efficacy. Several studies have demonstrated that methanolic and hydroethanolic extracts of *B. orellana* seeds and leaves exhibit notable inhibitory activity against both Gram-negative and Gram-positive bacteria. The antimicrobial ability of *B. orellana* L. has been attributed to its capacity to lyse microbial cell membranes and impair vital metabolic pathways, thereby suppressing microbial growth or causing cell death[1]. In addition, the antioxidant activity of *B. orellana* L. may further enhance its antimicrobial effectiveness by limiting oxidative stress and microbial survival[2].

*Withania somnifera* (L.) Dunal, commonly referred to as *Ashwagandha* or Indian ginseng, is another extensively studied medicinal plant that has been used in traditional Ayurvedic practice for thousands of years. While *W. somnifera* is widely acknowledged for its immunomodulatory and antioxidant properties, growing scientific evidence supports its antimicrobial potential as well. Various studies have reported that methanolic extracts of the leaves, roots, and stems of *W. somnifera* exhibit inhibitory activity against a range of bacterial and fungal pathogens. The antimicrobial activity of *W. somnifera* has been linked to the presence of bioactive compounds such as withanolides, flavonoids, alkaloids, and saponins, which may act individually or synergistically to inhibit microbial growth[3,4]. These findings highlight the potential of *W. somnifera* as a naturally occurring antimicrobial agent, in addition to its well-established therapeutic uses (Figure 1).

The concept of polyherbal formulation is a cornerstone of traditional medicine, where combinations of medicinal plants are believed to produce superior therapeutic outcomes compared with single-herb preparations. Polyherbal formulations offer several advantages, including enhanced antimicrobial efficacy through synergistic interactions, a broader antimicrobial spectrum, reduced toxicity, and improved formulation stability. The combination of *B. orellana* L. and *W. somnifera* is therefore of particular interest, as both plants possess complementary phytochemical profiles that may collectively enhance antimicrobial activity. Synergistic interactions between their bioactive constituents may result in improved microbial inhibition compared with individual plant extracts.

Topical drug delivery systems are widely preferred for the treatment of localised microbial infections owing to their ability to deliver active compounds directly to the site of infection, minimise systemic side effects, and improve patient compliance. Among various topical formulations, gels are especially advantageous because of their non-greasy nature, ease of application, good spreadability, and aesthetic appeal. Herbal gels formulated using suitable gelling agents such as Carbopol are known to provide



(A) (B)

**Figure 1.** Photographs of the study plants: **(A).** *B. orellana* L. leaves and **(B).** *W. somnifera* (L.) Dunal leaves.

excellent consistency, stability, and skin compatibility. Incorporation of medicinal plant extracts into a gel base not only enhances their therapeutic efficacy but also ensures prolonged contact with the affected area, thereby improving antimicrobial action.

Considering the growing demand for plant-based antimicrobial alternatives and the documented pharmacological potential of *B. orellana* L. and *W. somnifera*, the present study was designed to evaluate the antimicrobial activity of methanolic leaf extracts of these plants and to develop a polyherbal gel formulation. The study further aims to correlate antimicrobial efficacy with the phytochemical constituents present in the extracts, thereby providing scientific validation for their traditional use and supporting their potential application in safe, stable, and effective topical antimicrobial formulations.

## 2. Materials and Methods

### 2.1 Collection and Verification of Plant Material

Fresh green leaves of *B. orellana* L. and *W. somnifera* were collected from the campus of Shri Guru Ram Rai University, Dehradun, and nearby local areas. The plant material was washed thoroughly with distilled water to remove extraneous matter and dried in shade at room temperature. Botanical authentication was carried out by Dr. Anup Chandra, Head, Department of Botany, Forest Research Institute (FRI), Dehradun, as per authentication certificate no. 346/Dis./2018/Syst.Bot/Rev.Gen/4-5 and 2019/Dis./2018/Syst.Bot/Rev.Gen/4-5. Voucher specimens were deposited in the institute herbarium for future reference. Authentication and documentation followed standard taxonomical procedures[1].

### 2.2 Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade and were obtained from standard pharmaceutical suppliers; these included Methanol, Dimethyl Sulfoxide (DMSO), Carbopol-940, propylene glycol-400, methyl paraben, propyl paraben, sodium metabisulphite, and ofloxacin. Distilled water was used throughout the experimental procedures. Analytical-grade solvents and excipients are recommended to ensure reproducibility and accuracy in phytochemical extraction, antimicrobial assays, and gel formulation[5,6].

### 2.3 Preparation of Methanolic Extracts

The leaves of *B. orellana* L. and *W. somnifera* were dried in the shade and crushed into coarse powder using a mechanical grinder. Approximately 25 g of each powdered material was extracted with 250 mL methanol in a Soxhlet apparatus at 50-55°C until the extracting solvent ran colourless. The extract was then concentrated using a rotary evaporator and stored at 4°C until further analysis. Methanol was selected as the extraction solvent for its broad polarity range, which allows efficient solubilisation of phenolic compounds, flavonoids, and alkaloids.

### 2.4 Phytochemical Screening

The major secondary metabolites of the methanolic plant extracts were analysed by phytochemical screening. Qualitative tests were performed for alkaloids (Wagner's test), flavonoids (alkaline reagent and acid tests), glycosides (Keller-Killiani test), saponins (froth test), steroids and terpenoids (Salkowski's test), and tannins (ferric chloride and lead acetate tests). These standard phytochemical tests are widely employed for the preliminary characterisation of medicinal plant extracts[5,7].

### 2.5 Microorganisms and Culture Conditions

The antimicrobial potential of the extracts was examined against selected Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus agalactiae*), Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus*

*vulgaris*), and a yeast (*Candida albicans*). All microbial isolates were obtained from the Research Laboratory, School of Basic and Applied Sciences, SGRR University. Cultures were preserved on nutrient agar slants and subcultured periodically. Actively growing cultures were prepared by incubating the organisms in nutrient broth at 37°C for 24 h prior to experimentation[8].

## 2.6 *In Vitro* Antimicrobial Activity

The antimicrobial activity of *B. orellana* Methanolic Extract (BOME), *W. somnifera* Methanolic Extract (WSME), and the Polyherbal Methanolic Extract (PHME) was evaluated using the agar well diffusion method. Sterile nutrient agar plates were uniformly inoculated with microbial suspensions using sterile cotton swabs, and wells of 8 mm diameter were aseptically punched into the agar medium. Test solutions were prepared at concentrations of 0.1 mg/mL and 0.2 mg/mL using DMSO as the solvent. Ofloxacin (0.2 mg/mL) served as the standard drug, while DMSO served as the negative control. Plates were incubated at 37°C for 24 h, and zones of inhibition were measured (mm). All experiments were carried out in triplicate. The agar well diffusion assay is a widely accepted method for preliminary antimicrobial screening of plant extracts[1].

## 2.7 Preparation of Polyherbal Gel Formulation

The polyherbal gel was formulated using Carbopol-940 as the gelling agent. Carbopol-940 (2 g) was dispersed in distilled water with continuous agitation and allowed to hydrate completely. Propylene glycol-400 was incorporated as a humectant, and sodium metabisulphite along with parabens was added as preservatives. The required quantity of PHME was incorporated to prepare 2% and 5% w/w gel formulations. Triethanolamine was added dropwise to adjust the pH to skin-compatible levels. Carbopol-based gels are widely used for topical herbal formulations owing to their good stability, spreadability, and skin compatibility, which make them suitable vehicles for herbal actives[3].

## 2.8 Evaluation of Polyherbal Gel

The formulated gels were evaluated for physical appearance, colour, homogeneity, odour, washability, pH, and viscosity. A calibrated digital pH meter was used to measure the pH of a 1% aqueous solution of the gel at 25°C. Viscosity was determined using a Brookfield viscometer under controlled temperature conditions. Stability studies were conducted for one month under room temperature and accelerated storage conditions to assess changes in the physicochemical parameters. These evaluation parameters are essential for ensuring the quality and stability of topical gel formulations[9,10]. Together, these parameters constitute a standard and comprehensive physicochemical characterisation battery for a topical gel formulation, in line with established pharmacopoeia and industrial-pharmacy practice for herbal semisolid dosage forms[9,10].

## 2.9 *In Vivo* Pharmacological Activity

Healthy Wistar albino rats, each weighing between 180 and 200 g, were used for this study. The experimental protocol was approved by the Institutional Animal Ethics Committee in accordance with CPCSEA guidelines, India. The rats were acclimatised to controlled laboratory conditions maintained at 25 ± 2°C, with relative humidity of 44-56% and a 12:12 h light-dark cycle. Animals had unrestricted access to a standard diet and water throughout the study. OECD Guideline No. 402 was followed to evaluate the acute dermal toxicity of the polyherbal extract. Adult Wistar rats of both sexes were used, with nine animals divided into three groups of three rats each. Approximately 24 h prior to testing, 10% of the body hair from the dorsal area was removed using a depilatory agent (Veet). Group I served as the control, while Group II received a topical application of the polyherbal gel formulation (PGF, 5%) at a dose of 2000 mg/kg body weight. The rats were observed over 14 days for changes in fur, eye condition, behaviour, and skin reactions indicative of toxicity[11].

## 2.10 Grouping of Animals and Treatment Schedule

Experimental animals and their grouping are listed in Table 1.

## 2.11 *In Vivo* Excision Wound Model

An excision wound was created on the dorsal region of each rat. The dorsal area was shaved with a depilatory cream (Veet), and the rats were anaesthetised with

**Table 1.** Grouping of animals and treatment schedule

| Group No. | Treatment group | Treatment applied | Dose/concentration | Purpose |
|---|---|---|---|---|
| Group I | Normal Control | No treatment | — | To observe natural wound healing |
| Group II | Negative Control (Gel Base) | Carbopol gel base only | 500 mg/day (topical) | To assess the effect of gel base |
| Group III | Test Group I | Polyherbal Gel Formulation (PGF) | 2% w/w, 500 mg/day (topical) | To evaluate wound healing at a lower concentration |
| Group IV | Test Group II | Polyherbal Gel Formulation (PGF) | 5% w/w, 500 mg/day (topical) | To evaluate dose-dependent wound healing |
| Group V | Standard Control | Standard wound healing drug (*e.g.*, Povidone-iodine/ Ofloxacin gel) | As per standard dose | Compared with established therapy |

ketamine hydrochloride (100 mg/kg, administered intraperitoneally). An impression was made on the shaved area to mark the wound site, and a full-thickness excision wound with a circular area of 500 $mm^2$ was created using toothed forceps, a surgical blade, and pointed scissors. The wounds were left uncovered in an open aseptic environment[12,13]. Starting from the day of surgery (Day 0), 500 mg of the simple gel base, formulated extract gel, or standard drug was applied once daily until complete healing. To ensure precise application, the gels were weighed and divided into six equal parts, allowing each rat to receive 500 mg of the topical formulation daily; this protocol was followed for all treatment groups, including the gel base, 2% and 5% PGF gels, and the standard treatment. Wound area was measured by tracing the wound outline on Days 10, 20, and 30 for all groups, and wound contraction was expressed as a percentage of the healed wound area relative to the initial wound size, calculated using the following formula:

$$\% \text{ wound contraction} = [(\text{Initial wound area} - \text{Wound area on specific day})/\text{Initial wound area}] \times 100$$

## 3. Results

### 3.1 Phytochemical Analysis

Phytochemical screening of the methanolic extracts of *B. orellana* L. and *W. somnifera* is presented in Table 2.

### 3.2 Formulation of Polyherbal Gel

Representative agar well diffusion plates showing zones of inhibition of BOME, WSME, and PHME against the tested microbial strains are presented in Figure 2.

**Table 2.** Phytochemical screening of methanolic extracts of *B. orellana* L. and *W. somnifera* (L.) Dunal

| Phyto chemical Test | Test | *B. orellana L.* | *W. somnifera* |
|---|---|---|---|
| Alkaloids | Wagner's test | + | + |
| Flavonoids | Alkaline reagents | + | + |
| Glycosides | Keller-Killiani test | - | + |
| Saponins | Froth test | + | + |
| Steroids/ Terpenoids | Salkowski's test | + | + |
| Tannins | Ferric chloride test | + | + |

### 3.3 *In Vitro* Antimicrobial Activity

The antimicrobial activity of the methanolic extracts and their polyherbal combination at 0.1 mg/mL is shown in Table 3. Zones of inhibition were observed against all tested microbial strains.

The antimicrobial activity at 0.2 mg/mL is presented in Table 4. Increased zones of inhibition were observed at 0.2 mg/mL compared with 0.1 mg/mL for all extracts, and the standard drug exhibited the highest zones of inhibition among all tested groups.

### 3.4 Physicochemical Evaluation of Polyherbal Gel

The physicochemical properties of the formulated polyherbal gel are summarised in Table 5. The gel was clear, green in colour, and homogeneous. The pH was recorded as 6.58, and the viscosity was 6535 cps.

### 3.5 Skin Irritation Study

No erythema or oedema was observed in animals treated with the polyherbal gel during the 7-day observation period.

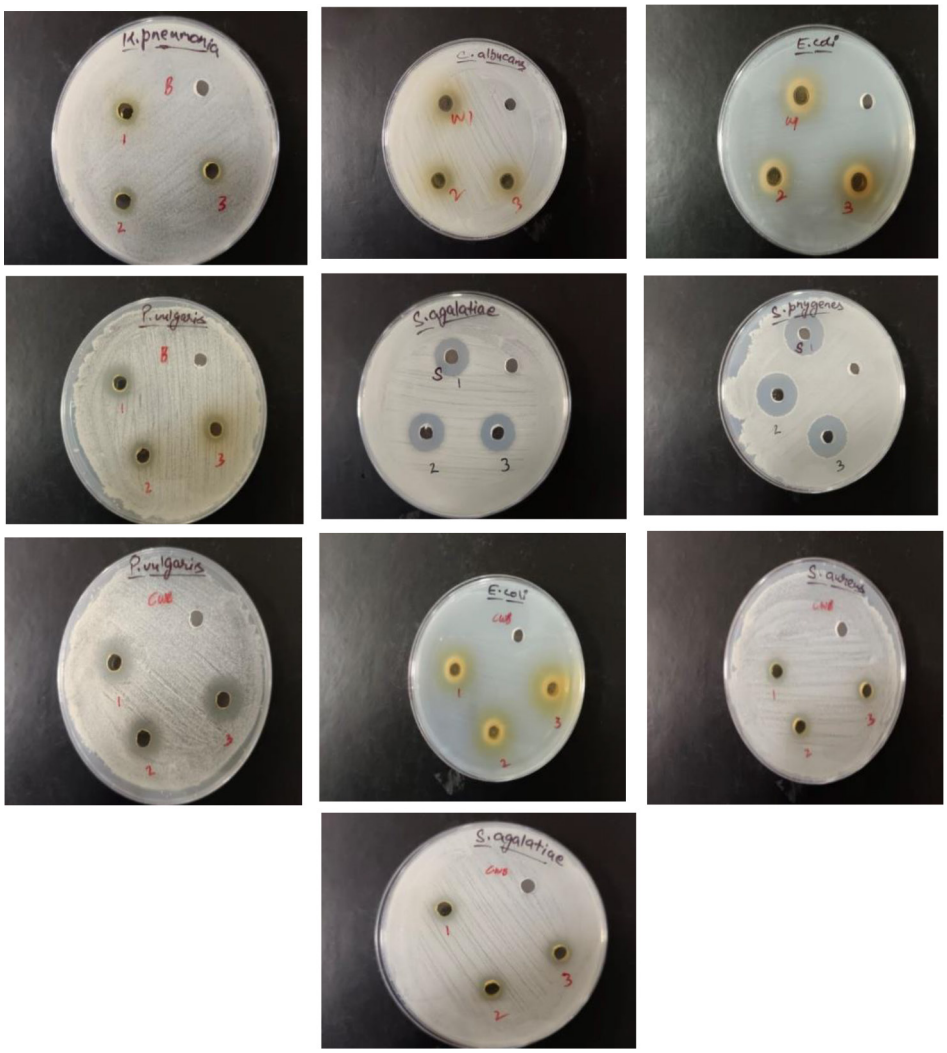



**Figure 2.** Representative agar well diffusion plates showing zones of inhibition of BOME, WSME, and PHME against the tested microbial strains.

**Table 3.** Antimicrobial activity (zone of inhibition, mm) of BOME, WSME, and PHME at 0.1 mg/mL

| Microbial Strain | *B. orellana* L. | *W. somnifera* | Combination | Standard |
|---|---|---|---|---|
| *E. coli* | 10±0.51 | 12±0.71 | 13±0.56 | 28±0.65 |
| *K. Pneumonia* | 12±0.53 | 7±0.75 | 10±0.57 | 25±0.68 |
| *C. albicans* | 11±0.52 | 10±0.74 | 12±0.58 | 28±0.64 |
| *S aureus* | 10±0.59 | 10±0.78 | 13±0.51 | 27±0.61 |
| *S. pyogenes* | 8±0.55 | 8±0.76 | 9±0.59 | 24±0.67 |
| *S. agalaetiae* | 8±0.59 | 9±0.76 | 10±0.58 | 28±0.65 |
| *P. vulgaris* | 10±0.57 | 6±0.78 | 11±0.53 | 25±0.64 |
| Mean±SEM | 9.85±0.55 | 8.85±0.76 | 11.14±0.59 | 26.42±0.64 |
| SD | 1.46385 | 2.03540098 | 1.573591585 | 1.718249 |

**Table 4.** Antimicrobial activity (zone of inhibition, mm) of BOME, WSME, and PHME at 0.2 mg/ml

| Microbial strain | *Brixa orellana L* | *W. somnifera* | Combination | DMSO | Standard |
|---|---|---|---|---|---|
| *E. coli* | 23.33±0.41 | 19.66±0.49 | 23.33±0.49 | 5±0.041 | 29.66±0.30 |
| *K. Pneumonia* | 22± 0.39 | 20.66±0.53 | 20.33±0.51 | 5±0.043 | 30.66±0.31 |
| *C. albicans* | 21.33±0.40 | 22.33±0.50 | 21.33±0.53 | 5±0.039 | 31.66±0.35 |
| *S. aureus* | 20.67±0.46 | 23± 0.51 | 23.33±0.51 | 5±0.041 | 31.66±0.29 |
| *S. pyogenes* | 23.66±0.43 | 21.33±0.53 | 23.66±0.53 | 4.66±0.42 | 30.66±0.30 |
| *S. agalaetiae* | 22.33±0.41 | 19.33±0.48 | 20.66±0.56 | 5±0.039 | 31.66±0.32 |
| *P. vulgaris* | 20.66±0.43 | 20±0.49 | 21±0.51 | 5±0.041 | 32±0.31 |
| Mean±SEM | 21.99±0.45 | 20.90±0.52 | 21.94±0.54 | 4.95±0.04 | 31.13±0.31 |
| SD | 1.20047 | 1.385032233 | 1.432264275 | 0.12851 | 0.836853517 |

**Table 5.** Physicochemical properties of the formulated polyherbal gel

| S. No. | Parameters | Results |
|---|---|---|
| 1 | Appearance | Clear |
| 2 | Color | Green |
| 3 | Homogeneity | Good |
| 4 | pH | 6.58 |
| 5 | Odour | None |
| 6 | Viscosity | 6535cps |
| 7 | Washability | Washable |

## 3.6 Stability Study

The formulation showed no change in physical appearance, pH, or viscosity under room temperature or accelerated storage conditions during the study period.

## 3.7 Acute Dermal Toxicity

No mortality or observable signs of toxicity were recorded at a dose of 2000 mg/kg. No behavioural or dermal abnormalities were observed during the study period.

**Table 6.** Percentage wound contraction on Days 10, 20, and 30 across treatment groups

| Treatment | 10 Days | 20 Days | 30 Days | Mean | SD | SEM |
|---|---|---|---|---|---|---|
| 2% PGF | 41.01** | 58.21* | 81.26* | 60.16 | 16.48974 | 9.520358 |
| 5% PGF | 55.56*** | 85.46** | 98.79** | 79.93667 | 18.07556 | 10.43593 |
| Gel Base | 37.55 | 50.55 | 75.57 | 54.55667 | 15.77805 | 9.109459 |
| Control | 25.4 | 47.8 | 68.27 | 47.15667 | 17.50752 | 10.10797 |
| Standard | 62.7*** | 88.97*** | 100*** | 83.89 | 15.6456 | 9.032993 |



**Figure 3.** Sequential photographs of wound healing progression on Days 0, 10, 20, and 30 across treatment groups.

### 3.8 Wound Healing Activity

The percentage wound contraction recorded on Days 10, 20, and 30 in the different treatment groups is presented in Table 6.

Representative photographs illustrating the progression of wound healing on Days 0, 10, 20, and 30 are shown sequentially in Figure 3.

## 4. Discussion

This study shows that *B. orellana* L. and *W. somnifera* methanolic leaf extracts, both alone and in combination, are antibacterial and support wound healing when formulated into a topical polyherbal gel. These findings support polyherbal formulations as a viable alternative or adjunct to conventional antibacterial and wound-healing therapies at a time of rising antimicrobial resistance.

### 4.1 Phytochemical Profile and its Biological Relevance

Preliminary phytochemical screening revealed that the methanolic Both extracts contained steroids, terpenoids, alkaloids, flavonoids, tannins, and saponins; glycosides were detected only in *W. somnifera*. These metabolites are known to have antibacterial, antioxidant, anti-inflammatory, and tissue-regenerative properties[5,7,14]. Methanol efficiently extracts phenolic and flavonoid compounds, which likely explains the biological activity observed here[15].

Flavonoids and tannins produce antibacterial effects by damaging microbial cell membranes, binding bacterial proteins, and inhibiting key enzymes; saponins act by increasing membrane permeability and causing microbial cell lysis[1,16].

The same compounds support wound healing by promoting angiogenesis, fibroblast proliferation, collagen synthesis, and epithelial regeneration.

### 4.2 *In Vitro* Antimicrobial Activity

Both the individual extracts and their polyherbal combination showed broad-spectrum activity against Gram-negative bacteria, Gram-positive bacteria, and *C. albicans.* At the lower concentration (0.1 mg/mL), the PHME produced larger zones of inhibition than either extract alone, indicating a synergistic or additive interaction between the phytoconstituents of *B. orellana* L. and *W. somnifera*. This kind of synergism, where multiple bioactive compounds act on several microbial targets at once, is a core principle of polyherbal therapy[1,17].

At the higher concentration (0.2 mg/mL), *B. orellana* L. extract alone matched the polyherbal combination against most strains, pointing to a substantial contribution from *B. orellana* L. to microbial suppression, consistent with other reports that *B. orellana* L. has strong antibacterial activity, attributed to terpenoids, flavonoids, and carotenoids (bixin and norbixin) that disrupt microbial cell walls and metabolism[2,18].

*Withania somnifera*'s antibacterial activity was comparatively mild, but its contribution to the mixture should not be discounted. Withanolides and alkaloids from *W. somnifera* are known to enhance immune regulation and reduce inflammation, which may indirectly support antimicrobial defence and tissue regeneration[4,15].

### 4.3 Physicochemical Properties and Safety of the Polyherbal Gel

The formulated gel had acceptable pH (6.58), viscosity, homogeneity, and stability at both room temperature and accelerated storage conditions. A pH close to skin pH is important for topical preparations, as it reduces irritation and supports wound healing[9,10]. No toxicity was observed in acute dermal toxicity testing, and no erythema or oedema was seen in skin irritation studies, supporting the formulation's cutaneous safety and biocompatibility.

### 4.4 *In Vivo* Wound Healing Activity

In the excision wound model, rats treated with the polyherbal gel showed significantly better wound contraction and faster epithelialisation than the control and gel-base groups. The 5% formulation performed best, matching the standard drug and indicating a dose-dependent response.

This enhanced healing likely reflects the combined antibacterial, antioxidant, and anti-inflammatory activity of the phytoconstituents: A lower microbial burden at the wound site prevents secondary infection, while antioxidant activity reduces oxidative stress and accelerates tissue regeneration[1,3]. Flavonoids and tannins also accelerate re-epithelialisation, improve

tensile strength, and promote collagen deposition, all critical to wound healing.

*Bixa orellana* L. alone healed wounds effectively, consistent with earlier work linking its phenolic compounds and carotenoid pigments to increased angiogenesis and fibroblast activity[2]. The addition of *W. somnifera* likely added to this effect, improving the overall healing response of the polyherbal formulation by lowering inflammation and promoting tissue remodelling.

### 4.5 Significance of the Polyherbal Approach

These results support the broader view that polyherbal formulations offer greater therapeutic benefit than single-plant remedies. The antibacterial synergy seen at lower concentrations and the improved wound healing at higher concentrations both demonstrate the advantage of combining plants with complementary pharmacological properties, which can lower the effective dose of each extract, limit toxicity, and broaden the therapeutic spectrum.

### 4.6 Limitations and Future Perspectives

These findings are promising, but further work is needed to quantify the active compounds, clarify the molecular pathways underlying the antimicrobial and wound-healing effects, and evaluate long-term safety. Spectroscopic confirmation of the key phytoconstituents, rheological profiling of the gel, and *in vitro* drug release studies would strengthen the formulation's characterisation, while cytokine profiling, histological analysis, and clinical trials would be needed to confirm its therapeutic potential for human use.

## 5. Conclusion

The current study demonstrates the antibacterial and wound-healing properties of *B. orellana* L. and *W. somnifera* methanolic leaf extracts, both independently and in combination. Phytochemical analysis revealed that *W. somnifera* contained secondary metabolites such as flavonoids, tannins, saponins, alkaloids, terpenoids, and glycosides, which most likely contribute to the reported biological activities. The favourable physicochemical qualities, stability, and cutaneous safety of the polyherbal gel formulation make it suitable for topical application.

The combined extract generally showed higher activity at a lower concentration in the *in vitro* antimicrobial studies, suggesting synergistic or additive effects among the plant constituents, with *B. orellana* L. appearing to be more influential at higher concentrations. In the *in vivo* wound-healing studies, the polyherbal gel showed enhanced wound contraction and epithelialisation, particularly at 5% concentration, with efficacy comparable to the standard drug.

*Bixa orellana* L. and *W. somnifera* were found to be non-toxic, non-irritant, stable, and safe under the experimental conditions tested, indicating their suitability for use in an antibacterial and wound-healing polyherbal topical formulation. The present study supports their pharmacological value. Further work should focus on identifying specific bioactive compounds, elucidating precise molecular mechanisms, and validating clinical efficacy for potential human use.

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## Author Contribution

Abhay Goswami: Investigation, Methodology, Data Curation, Writing – Original Draft, Rahul Sharma: Investigation, Methodology, Data Curation, Writing – Original Draft, Aarti Sati: Conceptualization, Supervision, Writing – Review and Editing, Preeti Juyal: Writing – Review and Editing, Priyanka Bhandari: Writing – Review and Editing, Suraj Chaudhary: Writing – Review and Editing, Sohit Saini: Writing – Review and Editing. All authors have read and agreed to the published version of the manuscript.