Preparation and Evaluation of Herbal Gel Incorporating *Saussurea obvallata* Extract with Antimicrobial and Wound Healing Potential

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ABSTRACT:
Saussurea obvallata (DC.) Edgew. Brahma Kamal (Asteraceae) is a plant species vulnerable to extinction from the Himalayan region, traditionally used for wound healing, and for antimicrobial use. The objective of the present study was to formulate topical gels of S. obvallata hydroethanolic leaf extract with Carbopol 940 and evaluate the physiochemical properties, antibacterial activity and physiochemical properties of the developed formulations and their potential for wound healing. The various concentrations of the extract (1%, 2%, 3% w/v) were converted to different gel formulations (F1, F2, F3) and their physical properties: appearance, pH, spreadability, viscosity, and skin irritation were assessed. Antibacterial activity was evaluated against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* by using the agar well diffusion method. A burn wound model in wistar rats has been used to assess the wound healing potential for 20 days. The phytochemical screening revealed the presence of flavonoids, tannins, phenolics, alkaloids, terpenoids, glycoside and saponins. The percentage of dried extract obtained from ethanolic extraction was 14.52% w/w. F2 (2% extract) exhibited physicochemical properties that were optimal with a pH of 6.34 ± 0.02, spreadability of 24.6 ± 0.42 g·cm/s and viscosity of 4785 ± 103 cP. The zone of inhibition for F2 against all the tested organisms was significant ranging from 13.85 to 16.85 mm. For burn wound model, F2 treatment resulted in 95.48 ± 1.16% wound contraction by day 20, which showed a similar epithelialization period of 17.40 ± 0.96 days compared to Povidone-Iodine standard group. It was not irritating in skin irritation tests. The results indicated that *S. obvallata* topical gel has the potential to be an effective antibacterial and wound healing agent, supporting its traditional applications and suggesting further research is warranted.

Keywords: Saussurea obvallata; Brahma Kamal; topical gel; wound healing; antibacterial; Carbopol 940; burn wound model; Himalayan medicinal plant.

Introduction

Wounds healing is one of the most complex process in the human body but it is still a significant clinical problem around the world. In developed nations, chronic wounds, such as diabetic foot ulcers, pressure ulcers, and venous leg ulcers, account for about 1-2% of the population, thus resulting in a significant economic burden estimated to exceed $25 billion per year in the United States alone [1,2]. It is expected that non-healing wounds will increase significantly as a result of the growing incidence of diabetes mellitus and progressive aging of the population across the globe [1–3].

Wound healing process is traditionally known as a series of 4 interrelated stages: hemostasis, inflammation, proliferation, and remodeling [4]. In chronic wounds, this well-balanced sequence is skewed and wounds cannot be healed because of a chronic inflammatory response, over-activity of matrix metalloproteinase (MMP) enzymes, impaired angiogenesis, and diminished growth factor signaling [1,4,5]. Today, wound management involves wound debridement, treatment of infection and the use of advanced therapies including bioengineered skin substitutes and preparations of growth factors; however, some of these advanced therapies are too costly or show inconsistent efficacy [4,6,7].

These restrictions have sparked renewed interest in the use of plant-derived wound healing agents that have multi-target activity, less expense, and a lower probability of antimicrobial resistance and fit into traditional medicine systems [8,9]. Saussurea obvallata (DC.) Edgew. Brahma Kamal (A. silihu, family Asteraceae), the lotus of Lord Brahma, is a perennial herb that is endemic to the Himalayas at elevations of 3,800–4,800 m. It is considered as the state flower of Uttarakhand, India and is also of great cultural and religious importance in the Himalayan region [8-10].

The use of the plant in Ayurvedic and Tibetan medicine systems has been long-standing with application to wound, cut, burn, respiratory, heart and urinary tract diseases [8,10,11]. Numerous modern pharmacological studies have identified a number of biologically active phytochemicals such as flavonoids, phenolic acids, terpenoids (particularly sesquiterpene lactones), alkaloids, tannins, saponins and steroids in its composition, which account for its antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, cardioprotective and radioprotective effects [8-10,12]. Although S. obvallata has been extensively used traditionally and has a good pharmacological profile, detailed assessment of the formulated topical preparations for wound management is scanty in the literature [8,9,13].

In recent years, the usefulness of hydrophilic gels based on Carbopol has been emphasized as the best vehicle for topical formulation used in wound healing because of their moist urine environment maintenance, high spreadability characteristics, compatibility with herbal extracts and sustained drug delivery when using a topical formulation [13-15]. In this context the present study was thus designed, for which, Carbopol 940 based topical gel formulations containing hydroethanolic leaf extract of *S. obvallata* were prepared and evaluated for their physicochemical properties, in vitro antibacterial activities and in vivo wound healing activities in a burn wound model in Wistar rats, providing scientific validation for the traditional use of this endangered Himalayan plant.

Materials And Methods

Materials

Carbopol 940 (pharmaceutical grade), propylene glycol, methyl paraben, propyl paraben, and triethanolamine were used as excipients. For antibacterial studies, Mueller–Hinton agar, nutrient broth and ciprofloxacin (positive control) were used. Ketamine hydrochloride was used for animal anesthesia. Unless indicated otherwise all chemicals and reagents were of analytical or pharmaceutical grade.

Plant Material Collection and Authentication

The leaves of Saussurea obvallata (DC.) Edgew. were collected from Balod district of Chhattisgarh, India in January 2026. The collected plant material was cleaned, washed with distilled water, shade dried for about 14 days, ground in a mechanical grinder and passed through sieve No. 40. The botanical authentication was done by Dr. Satish Kumar Sen, Botanist, V.Y.T.P.G. Autonomous College on 19/01/2026. The authenticated powdered material was kept in airtight amber containers at ambient temperature for further use [16].

Preparation of Ethanolic Extract

The leaves of S. obvallata were coarsely ground and extracted with 500 mL of 99% ethanol for 48 hours using Soxhlet process at 40–50°C, and then filtered. The extract was concentrated by rotary evaporation under reduced pressure and the dried mass was stored in a refrigerator at < 10°C in dark-coloured closed containers. The percentage yield was calculated as:

The percentage yield (%) was calculated as: (Weight of dried extract / Weight of powdered plant material) × 100 [13,17,18].

Preliminary Phytochemical Screening

Standard qualitative tests were done on the hydroethanolic extract for the detection of alkaloids (picric acid test), flavonoids (NaOH test), tannins (ferric chloride test), phenolic compounds (ferric chloride test), glycosides (Keller–Killiani test), steroids (chloroform/H_2SO_4 test), terpenoids (Salkowski test), saponins (froth test) and proteins (xanthoproteic test) using standard procedure [16].

Formulation of Topical Gel

Three formulations (F1, F2, and F3) of gel were prepared by using dispersion method, and using Carbopol 940 (1% w/v) as the gelling agent. The composition is shown in Table 1. Carbopol 940 was suspended in about 50 mL distilled water with continuous mechanical stirring and hydrated overnight. Methyl paraben and propyl paraben were dissolved in warm distilled water with addition of propylene glycol. The weighed quantity of S. obvallata extract was dispersed in propylene glycol by geometrical dilution and incorporated in Carbopol base under continuous stirring. Triethanolamine was added dropwise to assist gelation and to obtain pH 6.5–6.8. The final volume was made up to 100 mL with distilled water. The gels were placed in amber wide-mouthed glass containers [13,19].

Table 1. Composition of Saussurea obvallata Topical Gel Formulations

Ingredients	F1	F2	F3
Hydroethanolic extract (g)	1.0	2.0	3.0
Carbopol 940 (g)	1.0	1.0	1.0
Propylene glycol (mL)	10	10	10
Methyl paraben (g)	0.20	0.20	0.20
Propyl paraben (g)	0.02	0.02	0.02
Triethanolamine	q.s.	q.s.	q.s.
Distilled water (mL)	q.s. to 100	q.s. to 100	q.s. to 100

Evaluation of Gel Formulations

Physical appearance —All formulations were visually examined for colour, homogeneity, consistency, phase separation, and grittiness.

pH determination — One gram of gel was dispersed in 100 mL distilled water and pH measured using a calibrated digital pH meter in triplicate.

Spreadability — Spreadability (S) was determined using the slip and drag method: $S = M \times L / T$, where M = weight (g) tied to the upper glass slide, L = length traversed (cm), T = time taken (s).

Viscosity — Viscosity was measured using a Brookfield viscometer at room temperature.

Skin irritation study — The optimized formulation was applied to shaved dorsal skin of experimental animals; erythema and oedema were scored at 24 h [19].

In vitro Antibacterial Activity

The antibacterial activity was tested by agar well diffusion method against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* organisms involved in skin and wound infections [12].

Cultures were grown anew in sterile nutrient broth at 37°C for 18–24 h and adjusted to a turbidity of 0.5 McFarland standard (≈ 1.5 × 108 CFU/mL). The sterile Mueller-Hinton agar plates were uniformly inoculated and the wells of 6 mm diameter were bored with a sterile cork borer. Each formulation (100 µL) was loaded in respective well. Positive control: Solution of Ciprofloxacin. Blank gel base. Negative control. Plates were incubated at 37°C for 24h and zones of inhibition were measured in mm using a digital Vernier caliper. The procedure was modified from the procedure reported by Semwal and Painuli for topical gel evaluation [12].

In-vivo Wound Healing Activity

Experimental animals and ethics: Healthy adult Wistar albino rats of either sex weighing between 180–220 g were maintained under standard conditions (25 ± 2°C; 55 ± 5% RH; 12 h light/dark cycle) and provided with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) as per guidelines of the CPCSEA, Government of India.

Experimental groups: The rats were divided randomly into four groups of six animals each. Group I (disease control), Group II (Povidone-Iodine ointment), Group III (blank gel) and Group IV (optimized F2 gel).

Burn wound induction: Animals were anaesthetized with ketamine hydrochloride (50 mg/kg, i.p.). The dorsal fur was shaved, and the skin was disinfected with 70% ethanol. The dorsal thoracic skin was exposed to a cylindrical metal rod (≈2 cm diameter) heated in boiling water (80–90°C) for 5 min to produce a partial-thickness burn wound for ≈10 seconds under constant pressure [20].

Treatment and assessment: Topical treatment was started immediately after wound creation and continued once daily for 20 days. On days 0, 4, 8, 12, 16 and 20, the wound area was outlined on transparent sheets, transferred to graph paper and the percentage wound contraction was calculated as:

Wound contraction (%) = $[(A_0 - A_t)/A_0] \times 100$, where A_0 = initial wound area, A_t = wound area on the day of observation. The duration of complete epithelialization was also recorded [20].

Data Analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using GraphPad Prism software and the differences between groups were assessed by one-way ANOVA with Tukey's multiple comparison test. We used a p-value of < 0.05 for statistical significance [20].

Result

Percentage Yield

The ethanolic extraction of *S. obvallata* leaves yielded 14.52% w/w dried extract (Table 2), confirming appreciable quantities of ethanol-soluble phytoconstituents [13].

Table 2. Percentage Yield of Saussurea obvallata Extract

Parameter	Observation
Weight of dried powder	100 g
Weight of dried extract	14.52 g
Percentage yield	14.52 % w/w

Preliminary Phytochemical Screening

Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, tannins, terpenoids, glycosides, saponins, and steroids; proteins were absent (Table 3).

Table 3. Preliminary Phytochemical Profile of S. obvallata Extract

Constituents	Observation
Alkaloids	+
Flavonoids	+
Phenolics	+
Tannins	+
Terpenoids	+
Glycosides	+
Saponins	+
Steroids	+
Proteins	−

Physicochemical Evaluation of Gel Formulations

All three formulations appeared homogeneous and free from phase separation (Table 4). F2 exhibited superior homogeneity with a smooth consistency. Colour varied from light green (F1) to dark green (F3) with increasing extract concentration.

Table 4. Physical Characteristics of Gel Formulations

Formulation	Colour	Homogeneity	Consistency
F1	Light green	Good	Smooth
F2	Green	Excellent	Smooth

F3	Dark green	Good	Slightly viscous

pH values of all formulations were within the normal skin pH range (6.18–6.49), ensuring compatibility for topical application (Table 5). F2 demonstrated the best spreadability (24.6 ± 0.42 g·cm/s) and an optimum viscosity of 4785 ± 103 cP for topical use (Tables 6 and 7).

Table 5. pH of Gel Formulations (n = 3, Mean ± SEM)

Formulation	pH
F1	6.18 ± 0.04
F2	6.34 ± 0.02
F3	6.49 ± 0.05

Table 6. Spreadability of Gel Formulations (n = 3, Mean ± SEM)

Formulation	Spreadability (g·cm/s)
F1	22.4 ± 0.81
F2	24.6 ± 0.42
F3	18.7 ± 0.52

Table 7. Viscosity of Gel Formulations (n = 3, Mean ± SEM)

Formulation	Viscosity (cP)
F1	4250 ± 115
F2	4785 ± 103
F3	5325 ± 96

Skin Irritation Study

No erythema or oedema was observed with either the blank gel or the optimized F2 formulation (Table 8), confirming non-irritant potential of the formulation.

Table 8. Skin Irritation Scores

Group	Score
Blank gel	0
F2	0

Antibacterial Activity

All gel formulations demonstrated concentration-dependent antibacterial activity against all tested microorganisms, while the blank gel showed no inhibition (Table 9). F2 produced zones of inhibition ranging from 13.85 ± 0.42 mm (*P. aeruginosa*) to 16.85 ± 0.74 mm (*S. aureus*). **Figure 1** illustrates the antibacterial activity of the prepared gel formulations against *S. aureus*. The zone of inhibition increased with increasing extract concentration (F1 < F2 < F3), indicating concentration-dependent antibacterial activity. Although F3 exhibited the largest inhibition zone among the formulations, F2 was selected as the optimized formulation based on its superior physicochemical properties and wound-healing performance. Ciprofloxacin produced the highest antibacterial activity, whereas the blank gel showed no inhibition.

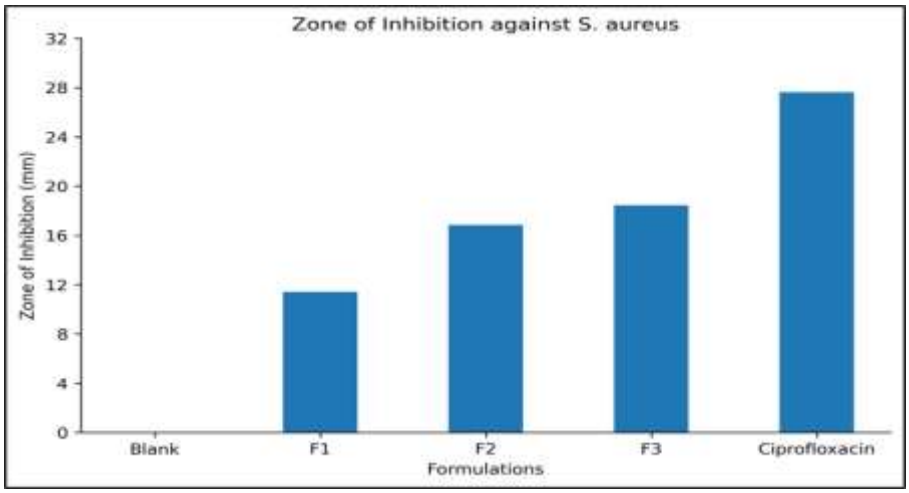

Figure 1. Zone of inhibition (mm) of *Saussurea obvallata* topical gel formulations (F1–F3) against *Staphylococcus aureus* compared with blank gel and ciprofloxacin (positive control). Values represent mean zone of inhibition measured after 24 h of incubation using the agar well diffusion method.

Table 9. Antibacterial Activity — Zone of Inhibition (mm, Mean ± SEM)

Test Organism	Blank Gel (mm)	F1 (1%) (mm)	F2 (2%) (mm)	F3 (3%) (mm)	Ciprofloxacin (mm)
S. aureus	0.00 ± 0.00	11.42 ± 0.53	16.85 ± 0.74	18.46 ± 0.62	27.64 ± 0.81
E. coli	0.00 ± 0.00	10.38 ± 0.44	15.26 ± 0.65	17.13 ± 0.58	26.32 ± 0.73
P. aeruginosa	0.00 ± 0.00	9.28 ± 0.36	13.85 ± 0.42	15.62 ± 0.54	25.84 ± 0.68
K. pneumoniae	0.00 ± 0.00	10.82 ± 0.41	14.93 ± 0.56	16.78 ± 0.61	26.11 ± 0.75

Wound Healing Activity

F2 significantly accelerated burn wound contraction compared with the disease control and blank gel groups. By day 20, the F2-treated group demonstrated 95.48 ± 1.16% wound contraction, comparable to the Povidone-Iodine standard group (98.65 ± 0.92%) (**Table 10 and Figure 2**). The F2 group also showed a significantly reduced epithelialization period (17.40 ± 0.96 days) vs. control (24.50 ± 1.18 days) (Table 11). **Figure 3** presents representative photographs illustrating the progression of burn wound healing in rats treated with the optimized F2 gel. A gradual reduction in wound area was observed throughout the treatment period, accompanied by scab formation, tissue regeneration, and progressive epithelialization. By day 20, nearly complete wound closure was evident, which is consistent with the quantitative wound contraction results presented in **Table 10** and the reduced epithelialization period reported in **Table 11**.

Table 10. Burn Wound Contraction (% Wound Contraction, Mean ± SEM; n = 6)

Days	Control (%)	Standard (%)	Blank Gel (%)	F2 (%)
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
4	14.25 ± 1.22	24.62 ± 1.36	16.48 ± 1.14	22.84 ± 1.28
8	28.74 ± 1.45	48.35 ± 1.54	31.28 ± 1.22	44.18 ± 1.43
12	46.81 ± 1.52	71.46 ± 1.71	50.62 ± 1.58	67.24 ± 1.64
16	62.38 ± 1.67	89.54 ± 1.52	66.15 ± 1.61	85.76 ± 1.42
20	78.42 ± 1.83	98.65 ± 0.92	81.46 ± 1.28	95.48 ± 1.16

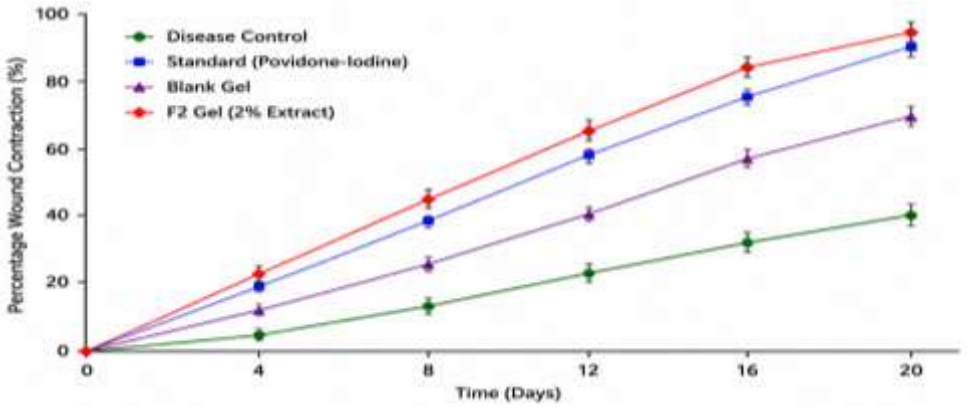

Figure 2. Time-dependent percentage wound contraction in experimental groups during the 20-day burn wound healing study. Values are expressed as mean ± SEM (n = 6).

Figure 3. Representative photographs showing burn wound healing in Wistar rats treated with the optimized F2 *Saussurea obvallata* topical gel on days 0, 4, 8, 12, 16, and 20, demonstrating progressive wound contraction and tissue regeneration.

Table 11. Epithelialization Period (Days, Mean ± SEM; n = 6)

Treatment Group	Epithelialization Period (Days)
Control	24.50 ± 1.18
Standard	16.20 ± 0.84
Blank Gel	22.60 ± 1.05
F2	17.40 ± 0.96

Discussion

The present study establishes the possibility of developing a topical gel based on Carbopol 940 using hydroethanolic extract of *Saussurea obvallata* with significant physicochemical, antibacterial and wound healing properties. The yield of ethanolic extract was 14.52% w/w. This value is comparable with the previously reported values of other related Saussurea species. It indicates that ethanol is an efficient solvent for the extraction of ethanol-soluble phytoconstituents [12,13]. Phytochemical screening showed the presence of flavonoids, tannins, phenolics, alkaloids and terpenoids, all classes with established roles in antimicrobial activity, anti-inflammatory modulation and promotion of tissue repair [8-10,12].

The physicochemical properties of the three formulations tested were most desirable with F2 (2% extract). The pH values of all the formulations (6.18-6.49) were within the physiological skin pH range, hence reducing the risk of skin irritation [13,14]. The low spreadability of F3 (18.7 ± 0.52 g.cm/s) compared to F2 (24.6 ± 0.42 g.cm/s) is explained by the higher viscosity due to the higher concentration of the extract. This is because the increased viscosity reduces the flow without a proportional increase in the therapeutic benefit. The ideal viscosity of a topical gel should be high enough to avoid run-off but at the same time it should have good spreadability. The viscosity of F2 was 4785 ± 103 cP which complies with both the requirements [13-15]. The non-irritant profile of F2 on skin irritation testing supports the safety of the formulation for topical application.

Antibacterial activity shows the concentration dependent inhibitory activity of S. obvallata gel formulations against Gram-positive bacteria (S. aureus) and Gram-negative bacteria (E. coli, P. aeruginosa, and K. pneumoniae). Despite F3 showing slightly larger inhibition zones, the differences were negligible and F2 was selected as the optimized formulation considering its better physicochemical performance. The antibacterial effects are consistent with previously reported activities of *S. obvallata* extracts, particularly against P. aeruginosa, K. pneumoniae, and S. aureus by Semwal and Painuli, and have been attributed to phenolic compounds, terpenoids, and flavonoids that disrupt bacterial cell membranes and inhibit enzyme systems [10,12]. The blank gel did not show any inhibition confirming the absence of contribution of excipients to the observed antibacterial activity.

The in vivo burn wound healing results strongly support the wound healing efficacy of F2 formulation. Animals treated with F2 achieved 95.48 ± 1.16% wound contraction by day 20 which was not significantly different from the Povidone-Iodine standard group (98.65 ± 0.92%) and significantly better than untreated controls (78.42 ± 1.83%) . The rapid contraction of the wound in F2 treated animals could be due to the synergistic action of the phytoconstituents. Antioxidant activity of flavonoids and phenolics reduces oxidative stress through free radical scavenging and protects healing tissues. Tannins cause protein precipitation and wound contraction, the terpenoids reduce production of inflammatory cytokine and protease activity, and the alkaloids have antimicrobial properties that reduce the microbial burden at the wound site [8-10,12]. These mechanisms in concert target major pathophysiological derangements in burn wound healing such as oxidative stress, infection, over-inflammation, and poor tissue regeneration [4,8].

The significantly reduced epithelialization period in F2-treated animals (17.40 ± 0.96 days vs. 24.50 ± 1.18 days in controls) further corroborates the wound healing efficacy of the formulation. Epithelialization is an expression of keratinocyte migration and proliferation, mediated by growth factors which are induced by anti-inflammatory and antioxidant phytoconstituents. These results are consistent with the wound healing potential of the zinc oxide nanoparticles synthesized using S. obvallata extract (84.70% wound closure) by Moalwi et al. and the nanoemulgel formulation of Bairagi et al. which showed complete epithelialization within 21 days [13,21]. The present simple gel formulation based on Carbopol is a more accessible and cost-effective approach and shows comparable efficacy, thus warranting further investigation including mechanistic studies and clinical evaluation.

Conclusion

The present study successfully demonstrates the development and evaluation of topical gel formulations based on Carbopol 940 containing hydroethanolic extract of leaves of Saussurea obvallata. Phytochemical screening revealed a rich arsenal of bioactive secondary metabolites of known wound healing and antimicrobial relevance. The formulation F2 (2% 2% w/v extract) showed best physicochemical properties (compatible pH, good spreadability and suitable viscosity) and was non-irritant. F2 showed significant broad spectrum antibacterial activity against clinically relevant wound pathogens and effectively enhanced burn wound healing in Wistar rats with around 95.48% wound contraction and epithelialization period similar to standard treatment. These findings provide important scientific evidence for the traditional use of S. obvallata in wound healing and point to the potential of this endangered Himalayan plant as a source of new and affordable wound healing therapeutics. Further studies on mechanism of action, optimization of nanotechnology based formulation, toxicological evaluation and clinical trials are needed to fully exploit the therapeutic potential of this promising medicinal plant.

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