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ORIGINAL ARTICLE

# Formulation and Evaluation of Wound Healing Activity of Sophorolipid-Sericin Gel in Wistar Rats

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## ABSTRACT

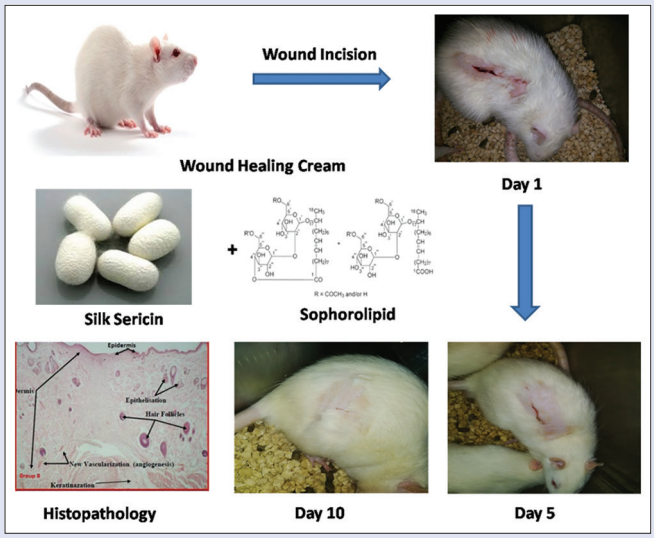
Sericin is a useful by-product of silk processing and is resistant to oxidation and ultraviolet and can absorb and release moisture easily. Sericin has biological activities such as antibacterial, antioxidant, and tyrosinase inhibition. Sophorolipids are microbial extracellular glycolipids produced by resting cells of *Candida bombicola*. Sophorolipids show excellent skin compatibility and also have antibacterial property. In this study, we have developed a novel formulation consisting of sericin and sophorolipid with calcium alginate as a binding agent. Since both the ingredients are biocompatible and biodegradable, the formulation was tested for wound healing in Wistar rats. A commercial ointment povidone was used as control. The animal group, treated with sericin and sophorolipid cream, showed fast contraction, rapid closure, and healing when compared with control and commercial ointment. These observations were validated with histopathological studies where more fibroblast proliferation, angiogenesis, and keratinization were observed. This is a green, cost-effective formulation for fast wound healing.

**Key words:** Biocompatibility, formulation, sericin, sophorolipid, wound healing

## SUMMARY

- A wound healing formulation was prepared using biocompatible compounds such as sericin and sophorolipid with sodium alginate as a binding agent. Sericin is a by-product/waste from textile industry which is readily available and a cheap source. Since all the ingredients are biocompatible, it is a green formulation and has no side effects. Formulation has showed viscosity, pH, spreadability, and extrudability in acceptable range. The formulation showed antioxidant activity. The treatment of rat wounds with sericin + sophorolipid cream has led to reduce scar formation and enhanced fibroblast proliferation, angiogenesis, keratinization, and epithelialization as compared other groups.

**Abbreviations used:** UV: Ultraviolet; $Na_2SO_4$: Sodium sulfate; RPM: Revolutions per minute; MGYP: Malt extract glucose yeast extract peptone; DPPH: 1,1-diphenyl-2-picrylhydrazyl; Abs: Absorbance; CPCSEA: Committee for the purpose of control and supervision of experiments on animals; IAEC: The Institutional Animal Ethics Committee; HPMC: Hydroxypropyl methylcellulose; SL: Sophorolipid

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## INTRODUCTION

Wound healing is a complicated process in which the skin repairs itself after injury. The normal wound healing process can be broadly classified into four stages, namely the hemostasis, inflammatory, proliferative and maturation phases.[1] In the early stage of wound healing, a fibrin clot is produced at the site of the wound in a moment following injury, fibrinogen is cleaved into fibrin monomers by thrombin, and the peptide monomers are polymerized by factor XIII. The resulting fibrin acts as a scaffold for different cells to move about in and out of the wound bed. Five to seven days later than the initial injury, fibroblasts migrate to the wound site, secreting new collagen, and keratinocytes migrate from the wound edge and shape a thin epithelial cell layer to secure the wound.[2] The inflammatory phase that lasts up to 2 days involves an orderly recruitment of cells to the wound area, which is followed by proliferative phase lasting up to 6 days. However, certain wounds are problematic and do not follow the normal timetable for the healing process and take a longer time to heal. Re-epithelialization is a critical step in wound healing, in which epidermal keratinocytes laterally migrate to close a wound. However, in chronic wounds, keratinocyte migration is blocked and the wounds remain open. Further, most wounds heal rapidly and efficiently, but the results are not perfect, as the healing process leaves a scar on the



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skin. Scar tissue is less flexible than normal skin and can be cosmetically disfiguring affected area. Two essential goals of wound healing (tissue repair) which includes rapid healing and complete reconstruction of the damaged area without leaving a scar.

Sericin protein is useful because of its special properties, namely antioxidant, antibacterial, and ultraviolet (UV) resistant, absorbs and releases moisture easily, and inhibits the activity of tyrosine kinase.[3] Sericin is also biocompatible and biodegradable in nature. The most important feature of the silk protein is that the dipeptides and tripeptides can easily permeate into the bloodstream through the dermis layer of skin.[4] Aramwit and Sangcakul[5] evaluated the effects of sericin on wound healing and wound size reduction using rats. Along with 8% sericin cream, they used Betadine as a control. They found that sericin-treated wound-size reduction was much greater than in the control. Aramwit *et al*.[6] claimed the first report of demonstrating that sericin is safe and useful in the treatment of burn wounds and confirmed that sericin can promote the healing of open wound as shown by the scratch assay. Efforts are being made all over the world to discover agents that can promote fast wound healing and thereby reduce the cost of hospitalization and save the patient from other severe complications. The need for new therapeutics for wound healing has encouraged the drive to examine the nature and value of silk products.[7]

Sophorolipids are glycolipid type of biosurfactant. The chemical composition of sophorolipid is constituted by a disaccharide sugar, namely sophorose and a fatty acid or an ester group. Different biosurfactants possess antimicrobial property. Various biosurfactants, for example, from *Bacillus circulans*, *Bacillus subtilis*, *Bacillus licheniformis*, *Candida antarctica*, and *Pseudomonas aeruginosa*, have been reported to have potent antimicrobial activity. By its structure, biosurfactant is supposed to exert its toxicity on the cell membrane permeability as a detergent-like effect.[8] Biosurfactants are coming up as emerging class of biomedical compounds. They are a suitable alternative to synthetic medicines and antimicrobial agents and could be used as safe and effective therapeutic agents.[9] There are no reports on the combined use of sericin and sophorolipids in wound healing preparations; therefore, here, we have attempted to determine whether sophorolipid can improve wound efficiency of sericin.

# MATERIALS AND METHODS

## Materials

Silk cocoons (*Bombyx mori*) were purchased from Sai Silk Industries, Jejuri, Pune, Maharashtra, India. Sophorolipid was produced by a nonpathogenic yeast *Candida bombicola* ATCC 22214 supplementing with oleic acid and glucose[10] (Joshi-Navare *et al*., 2011). Sodium alginate was obtained from Sigma Aldrich. Cipladine 5% Ointment, manufactured by Cipla Ltd. (Composition: Povidone Iodine) (povidone-iodine) was purchased from local market.

## Formulation of wound healing cream

Raw Chinese bivoltine silk fibers of *B. mori* were degummed using a fungal alkaline protease. Sericin was visible as insoluble sediment at the bottom, in the degumming liquor. The sediment was separated by centrifugation at 1000 rpm for 5–10 min. Sericin pellet (insoluble sericin) was washed with deionized water to remove soluble sericin as well as impurities. The insoluble portion was lyophilized and used for the preparation of wound healing formulation.

For Sophorolipid (SL) production, seed culture was prepared by inoculating 10 mL of fresh MGYP nutrient medium with *C. bombicola* ATCC 22214 followed by incubation at 30°C, 180 rpm for 24 h. This preinoculum was added to 90-mL MGYP nutrient medium in a 500-mL Erlenmeyer flask and incubated further for 48 h. Cells were harvested and washed twice with sterile distilled water. The cell pellets (biomass ~1.5-g dry weight in 100-mL medium) were redispersed in 100 mL of 10% glucose solution supplemented with 1 mL of oleic acid (dispersed in 1-mL ethanol), and again, incubation was continued for 96 h when a brown and viscous SL mass was seen settled at the bottom of the flask. It was separated using a pipette tip cut at nozzle and subjected to ethyl acetate extraction. Culture medium was centrifuged at 5000 rpm, at 10°C for 20 min. The supernatant was extracted twice with equal volumes of ethyl acetate, the organic layer was dried over anhydrous $Na_2SO_4$, and the solvent was removed by rotary vacuum evaporation. The yellowish brown semi-crystalline product was washed twice with n-hexane to remove unconverted fatty acid.[10]

To optimize the concentration of gelling agent to achieve proper consistency, the formulations were prepared with different gelling or thickening agents, various gums, carboxymethylcellulose sodium, sodium alginate, hydroxypropyl methylcellulose (HPMC), and different concentration of viscosity enhancer as 1%–8% were tried, and finally, cream that showed good spreadability and consistency was selected. The formulations contained sericin, sophorolipid, and sodium alginate as per the concentrations. Gelling agent sodium alginate was slowly added to the sericin with continuous stirring and heating on water bath (temperature: 60°C–70°C). Sophorolipid was added to it and stirred continuously till a uniform cream was formed after 15–20 min. The formulation/cream was then placed in UV light for 20–25 min and stored in plastic container at room temperature.

## Evaluation of cream

### *Physical examination*

The prepared formulations/creams were inspected visually for their color and homogeneity. The spreadability ($n = 3$) of the formulations was determined by measuring the spreading diameter of 1 g of cream between two horizontal plates (20 cm × 20 cm) after 1 min. The standardized weight tied on the upper plate was 125 g.

### *pH determination*

The pH was measured, at room temperature, using digital pH meter which was calibrated before each use with standard buffer solutions. The pH of the formulations was checked at 1, 10, 45, and 60 days after preparation to detect any pH changes with time.

### *Viscosity*

The measurement of viscosity of the prepared creams was done with a NDJ-1 viscometer. The creams were rotated at 6 and 12 rpm using spindle number 3. At each speed, the corresponding dial reading was noted.

## 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was carried out according to Takechi *et al*.[11] About 0.5 mg of DPPH was dissolved in 12 ml of methanol to make 1 mM of working solution. One gram of sample with varying concentrations was added with 1 ml of 1 mM DPPH. The mixture was incubated in the dark at room temperature for 30 min. Later, the absorbance was measured at 517 nm with DPPH and methanol as blank. Percentage of antioxidant activity was calculated as follows:

$$\% \text{ Antioxidant} = \frac{\text{Abs of blank} - \text{Abs of sample}}{\text{Activity Abs of blank}} \times 100$$

## *In vivo* wound healing model

*In vivo* experiments were performed at Symbiosis School of Biomedical Sciences, Lavale, Pune. Wister rats (male) weighing 250 ± 20 g were used

and all the studies were performed as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (CPCSEA Reg. No. SSBS/AH/04-2015). The Institutional Animal Ethics Committee (IAEC) study approval number was SSBS/IAEC/04-2015. The animals were housed in a standard individual metal cages, and room was maintained at temperature 22°C ± 1°C and relative humidity 55% ± 5% with an alternating 12 h light–dark cycle. Food and water were provided *ad libitum*. All the experiments on animals were conducted after obtaining permission from the IAEC of the institute.

## Pharmacological evaluation

Pharmacological evaluation was carried out with incision wound model. Animals were divided into four groups (six animals each), namely A – povidone, B – sericin and sophorolipid formulation, C – only sericin, and D – untreated. Body weights of the animals were in the range of 240–270. All animals of four groups were anesthetized with anesthetic ether, and a paravertebral long incision of 4.4 cm length was made through the skin and cutaneous muscle at a distance about 1.5 cm from the middle on the right side of the depilated back.

All groups of animals were received sufficient amount of preparation applied externally. All the test formulations were applied once a day for 10 days starting from the day of incision. Wound healing property was evaluated by wound length and wound closure time. The wound area was measured immediately by placing a transparent paper over the wound and tracing it out on every alternate day.

## Histopathological studies

Skin specimens from treated and untreated rats were collected in 10% buffered formalin, and after the usual processing, 5-mm-thick sections were cut and stained with hematoxylin and eosin.[12] Sections were qualitatively assessed under the light microscope and graded with respect to fibroblast proliferation, collagen formation, epithelialization, keratinization, and scar formation.

# RESULTS

## Formulation of the wound healing cream

To optimize the concentration of gelling agent to achieve proper consistency of the sericin–sophorolipid cream, formulations were prepared with different gelling or thickening agents, various gums, carboxymethylcellulose sodium, sodium alginate, and HPMC. Different concentrations of above-stated ingredients were checked for their property of gelling and viscosity builder. Concentrations used range from 1 to 10 g%. Among all above agents, sodium alginate with 2%–3% (w/v) showed good spreadability and consistency.

The formulations containing sericin and sericin + sophorolipid were brown in color. pH of only sericin containing formulation and sericin + sophorolipid was 6.1 and 5.6, respectively, which are compatible to our skin. Formulation containing sericin and sophorolipid containing cream showed better viscosity and spreading diameter than that of only sericin containing cream.

## Antioxidant activity

DPPH is a stable-free radical that shows maximum absorbance at 517 nm in methanol. When DPPH radicals encounter a proton-donating substance such as an antioxidant, the DPPH radicals would be scavenged and the absorbance is reduced.[13] Based on this principle, the antioxidant activity of the substance can be expressed as its ability in scavenging the DPPH radicals. Figure 1 shows that different concentrations of formulation (silk sericin + sophorolipid) possessed different abilities to quench the DPPH radicals. It was observed that 50–500 µg/ml of formulation scavenged 8.2%–76.1% of DPPH radicals, respectively. Antioxidant activities of silk sericin from silkworm *B. mori* were also evaluated and reported by Fan *et al.*[14] They also observed that different concentrations of silk sericin possessed different abilities to quench the DPPH radicals.

## *In vivo* wound healing model

An incision wound healing model followed to study the wound healing potential of the sericin and silver nanoparticles as active ingredients of the formulation. It took 8–10 days for complete healing of wounds of the test group. Photographic comparison and histopathological evaluation of the tissues are shown in the following points. Monitoring of inflammatory mediators induced by silk sericin evaluated on incision wound model by Aramwit and Sangcakul.[5]

## Photographic comparison

After creating a wound according to the guidelines of the CPCSEA, photographs of each group of animal's wounded part were taken for the visual comparison, and the same is shown in Figure 2. Following formulations applied to the wounds of each group of animals.

a – povidone formulation
b – sericin and sophorolipid formulation,
c – sericin formulation
d – untreated.

Figure 3 shows that formulation containing sericin and sophorolipid, i.e., Group B shows faster rate of wound contraction and healing process as compared to commercial preparation used in this study. It is also clearly seen that the addition of sophorolipid to sericin has rapid and faster healing. On 7$^{th}$ day approximately 90% of the wound was healed in the Group B animals and on 10$^{th}$-day complete healing observed in the visual examination. These results are in agreement with Aramwit *et al.*[6] where they added sericin to a standard antimicrobial cream (silver zinc sulfadiazine) and evaluated wound healing efficacy in comparison with the control cream. They observed that re-epithelialization and wound healing time with the sericin-added cream were significantly shorter. Padol *el al.*[15] used commercially available silk proteins with modifications for wound healing and reported wound healing in 15–20 days. Aramwit and Sangcakul[5] showed that sericin cream showed the wound healing after 11 days. These results suggest that addition of sophorolipid to sericin improves the wound healing rate.

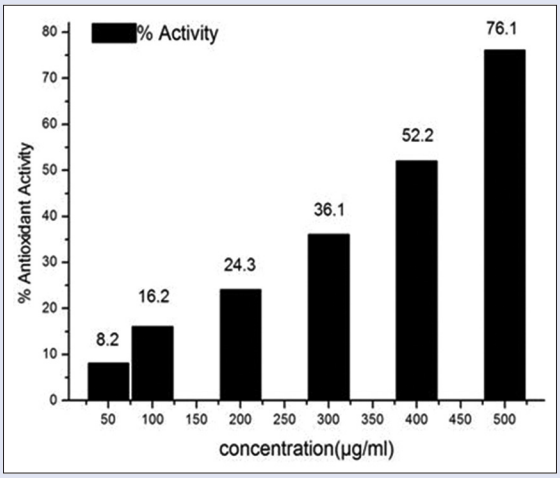


**Figure 1:** Percentage of antioxidant activity at different concentration of formulation

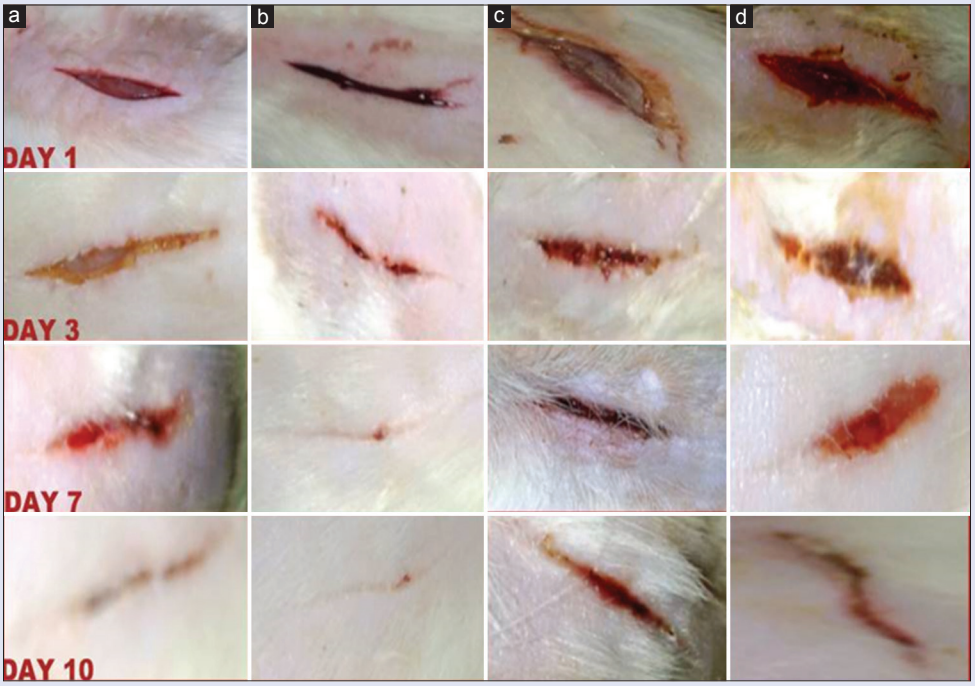


**Figure 2:** Photographic comparison of wounds. (a) Povidone, (b) sericin and sophorolipid formulation, (c) only sericin, and (d) untreated

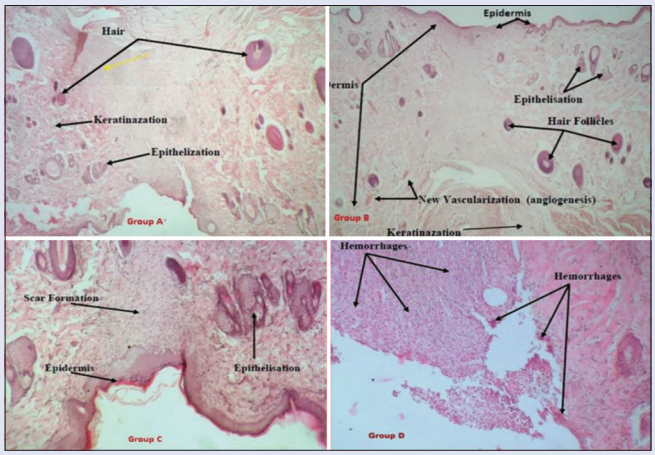


**Figure 3:** Histopathological evaluation of skin of rat, Group A (treated povidone-iodine), Group B (treated with sericin + SL), Group C treated with (sericin), and Group D (untreated)

## Histopathological evaluation

Histological examination was performed on skin art of wound that has been fixed after sacrifice of the exposed animal, and changes in the tissue and cell morphology were assessed using a light microscopy. The treatment of rat wounds with sericin + sophorolipid formulation led to reduce scar formation and enhanced fibroblast proliferation, angiogenesis, keratinization, and epithelialization as compared other groups. The rapid healing and contraction of wound in the treatment group animals may possibly be attributed to the increase in total protein and collagen content in the wound granulation tissue. Fibroblast proliferation, angiogenesis, and keratinization are more and faster in sericin + sophorolipid-treated animals as compared to the group treated with standard compound. About 10% formalin solution-fixed skin tissue of animals examined microscopically and revealed in Group D. The tissues were marked with exudates which are degenerating inflammatory cells, very slight epithelialization characterized by a few immature epithelial cells along with inflammatory cell infiltration, and found no fibroblast proliferation, incomplete epithelialization, congestion of vessels in the dermis, proliferation of fibroblasts, and infiltration of inflammatory cells were noticed, but there was no keratinization. The wounds of Group B treated with silk sericin with sophorolipid were microscopically revealed, it showed epithelialization with formation of epithelial layer, keratinization was observed, and the dermis showed neovascularization, matured fibroblasts, increased collagen synthesis, and absence of inflammatory cells. In addition, Group A treated with povidone-iodine showed regular arrangement of fibroblasts in the dermis. Severe hemorrhages and marked neovascularization are seen in the negative control Group D [Figure 3].

## CONCLUSION

It can be concluded that a new biocompatible wound healing cream will have a potential use for faster and scar-free wound healing.

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## Conflicts of interest

There are no conflicts of interest.

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