INTRODUCTION

Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological disturbances to the tissue. Wound healing is a biological process that is initiated by trauma and often terminated by scar formation. Thus, wound healing is an attempt to maintain normal anatomical structure and function of skin. Wound healing process occurs in different phases such as coagulation, epithelization, granulation, collagen formation, and tissue remodeling. Effective wound management promotes hemostasis and healing, prevents infection transmission and further injury, and maintains skin integrity. Skin and wound management must be individualized - considering both patient and skin/wound condition - to achieve a positive outcome. It is natural phenomenon where body itself overcomes the damage to the tissue. Various animal models are available to understand basic process of tissue repair and to study strategies for the treatment of wounds.[3]

Open wounds are prone to bacterial infection and also provide a way for systemic infections. Once infected, wound take longer time to heal. Furthermore, wound with infection leads into the formation of unpleasant exudates and toxins that further disturbs the formation of regenerating cells. Thus, wound healer stimulates healing and restores the normal functions of the affected part of the body to ease the discomfort and pain associated with wounds, preventing infection, and activating tissue repair processes.[2]

Majority of the developing countries are still dependent on herbal medicines for their primary health-care needs. All traditional systems of medicine, Ayurveda, Siddha, and Unani use plant, components, mineral, and compounds of animal origin for wound healing. Due to lesser side effect, abundance and easy availability plant-based product have gained much popularity. Many plants have been proved which can be effectively and safely used as wound healers.[3]

Antimicrobial and wound healing activity of Hibiscus rosa-sinensis Linn.

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ABSTRACT

Aim: Hibiscus rosa-sinensis Linn. is an medicinally and cosmetically important herb in Ayurveda. Wound healing activity of flower of H. rosa-sinensis L. is reported in Ayurvedic literature. However, scientific data are not available proving its use. Antibacterial and healing compounds of natural origin can be the choice of treatment in wounds. Hence, the present study was aimed to evaluate wound healing and antimicrobial potency of hydroalcoholic H. rosa-sinensis L. (HA-HRS) extract gel formulation. Materials and Methods: Herbal gel formulation containing 5% and 10% HA-HRS extract was prepared and optimized for spreadability viscosity and extrudability. In vitro antimicrobial activity of extract and gel formulation showed comparable result to that of povidone-iodine standard. Wound healing potential of formulated gel was studied using excision and incision wound model. Results and Discussion: Gel having 10% HA-HRS showed complete healing compared to the control (22.41%) after the 14th day. Histopathological observation in incision model stained with hematoxylin and eosin stain showed fibroblast proliferation in dermis and increased epidermal thickness for animals treated with 10% of extract containing gel and showed normal skin histology with dermis, epidermis, and hair follicle for excision model animals. Masson trichrome staining examination revealed moderate increase in collagen content at wound increase in collagen count at wound area for both the formulations in both the models. Conclusion: Gel containing 10% HA-HRS extract can be used as good alternative to synthetic wound healers.

KEY WORDS: Antimicrobial activity, Gel formulation, Hibiscus rosa-sinensis L., Wound healing activity

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Antibacterial and healing compounds from natural origin can be a better choice of treatment in wounds as compared to available synthetic drugs.

_Hibiscus rosa-sinensis_ Linn. belongs to the family Malvaceae, colloquially known as Chinese hibiscus or China rosa, is a specific of tropical hibiscus with attractive and colorful flowers, plants of Hibiscus are widely planted as ornamentals.\[4\] It is used in traditional medicine for the treatment of skin disease, as an antifertility agent, antiseptic, carminative, antimicrobial, antioxidant, antiviral, hypotensive, depressant, anti-inflammatory, analgesic, antipyretic, antitumor, and anti-implantation.\[6,7\] Similarly, flowers also have medicinal value as emollient, demulcent, refrigerant, and aphrodisiac and have cosmetic use to stimulate hair growth, prevent hair loss, premature graying, etc.\[8\] Flower preparation proves the antibacterial potential of _H. rosa-sinensis_ L.\[8\] Many chemical constituents have been reported.\[9-12\]

Conventionally, it is also used in wound healing. Very less scientific data are available in literature to prove its wound healing activity.\[13\] Hence, the present study was aimed with exploration of antimicrobial and wound healing activity of _H. rosa-sinensis_ L. extract gel formulation.

**MATERIALS AND METHODS**

**Plant Collection**

Fresh flowers of _H. rosa-sinensis_ L. (Malvaceae) were procured from local market Pimpri, Pune, Maharashtra. Plant was identified and authenticated by Botanical Survey of India, Pune, where a specimen has been kept. Flowers were washed cleaned for any foreign matter and were kept for drying in shade. After complete drying, flowers were crushed and grinded to from coarse powder.

**Preparation of Extract**

Flower powder (100 g) was packed in Soxhlet apparatus with thimble clogged with cotton to avoid transfer of sample particles to the distillation tank. This packed material was extracted at 60°C–70°C temperature with ethanol: water (7:3) mixture for 2 days and until clear solution was observed in siphon tube. The extract was concentrated under vacuum to get solid crude mass, and percent yield of extract was calculated hydroalcoholic _H. rosa-sinensis_ L. (HA-HRS).

**Proximal Analysis**\[14,15\]

Proximal analysis was carried out according to the procedure of The Ayurvedic Pharmacopoeia of India (Part - I, Volume - I). The powder was studied for total ash, acid insoluble, water soluble, loss on drying, and extractive value.

**Preliminary Phytochemical Study**\[14\]

Preliminary phytochemical study of HA-HRS extract was performed qualitatively for different phytochemical investigation by performing various qualitative chemical tests.

**Quantitative Phytochemical Analysis**\[16,17\]

**Total phenols content**

Total phenolic compounds in HA-HRS extract were determined by Folin–Ciocalteu’s calorimetric method (Waterhouse, 2002) using gallic acid as standard.

**Total flavonoids content (TFC)**

The flavonoids content in HA-HRS extract was determined spectrophotometrically (Kumar et al., 2008) using quercetin as standard.

**Method for Preparation of Gel Containing HRS-HA Extract**\[19-21\]

1 g of Carbopol-940 was dispersed in 50 mL of distilled water with continuous stirring and was kept overnight to swell. To 2 mL of distilled water, optimized quantity of methylparaben and propylparaben was dissolved by heating on water bath. The solution was cooled and propylene glycol 400 was added gradually to form a homogenous mass. Further required quantity of HA-HRS (10 mg and 5 mg) was mixed to the above mixture and volume was made up to 100 mL by adding remaining part of distilled water. All the ingredients were mixed properly with Carbopol-940 to form a smooth gel with continuous stirring. Finally, triethanolamine was added dropwise to adjust pH of 6.8–7.0. Same procedure was followed for preparation of control gel sample without adding extract. 10% HRS gel and 5% HRS gel were evaluated for consistency, viscosity, spreadability, and extrudability.

**Antimicrobial Susceptibility Test of Extract**

The antimicrobial activity of HA-HRS extract and HA-HRS (10% and 5%) gel was tested by agar disc diffusion method against _Staphylococcus aureus_ and _Escherichia coli_, and previously, liquefied medium was inoculated with 0.2 mL of bacterial suspension having a uniform turbidity. The culture medium (20 mL) was poured into the sterile Petri dish. Care was taken for the uniform spreading of the medium. After complete solidification of culture medium, the wells were produce by using barrier of diameter 6 mm. The wells were impregnated with the test material. The concentration used for the extract was 5 mg in 10 mL distilled water. Povidone-iodine ointment (5 mg/10 mL) was used as an antimicrobial agent.

Aseptically placed on the inoculated plates and the plates were left at ambient temperature for 30 min to allow pre-diffusion before incubation at 37°C for 24 h.
The antibacterial activity was estimated by measuring the diameter (mm) of the zone of inhibition.[18]

**Animal Study**

**Skin irritation study**

Nearly 10% HRS gel and 5% HRS gel were applied to an area of approximately 5 cm² of skin and covered with gauze patch. The gauze was loosely held in the area for 1 h. After 1 h, gel was removed. The gel was applied to the skin once in a day for 7 days and was observed for any sensitivity and reactions.

**Wound healing activity**[22-25]

Wistar albino rats (150–250 g body weight) of either sex were selected. They were acclimatized for a period of 7 days to the laboratory environment. They were housed individually in polypropylene cages at 23°C ± 1°C in 12:12 h dark: light cycle, with free access to standard pellet feed and water.

**Excision wound model**

Rats were anesthetized with anesthetic ether before the creation of wounds. The dorsal fur of the animals was shaved with razor. Excision wound of circular area of 300 mm² and 2 mm depth was created using toothed forceps,[23] a surgical blade, and pointed scissors. Homeostasis was achieved using cotton swab soaked in saline solution. The wound was kept open. The animals were treated as per their group once a day until complete epithelization occurred. The wound closure rate was calculated by tracing the wound area on days 0th, 2nd, 4th, 6th, 8th, 10th, 12th, and 14th post-wounding. Furthermore, the number of days required for epithelization was also calculated [Table 1].

\[
\text{% of wound closure} = \frac{\text{wound area on day } n}{\text{wound area on day zero}} \times 100
\]

Where, \( n \) = number of days 0th, 2nd, 4th, 6th, 8th, 10th, 12th, and 14th day.

**Incision wound model**

Rats were anesthetized with anesthetic ether before the creation of wounds. The dorsal fur of the animal was shaved with razor. Two paravertebral long incisions of 6 cm length were made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the shaved back of the rats. After the incisions made, the parted skin was kept together by stitching at 0.5 cm intervals continuously and tightly using surgical thread (No. 000) and a curved needle (No. 11). Wound was kept open. Animals were treated with extract (5% and 10% gel) and standard once a day. Wound breaking strength was calculated [Table 1].[25]

\[
\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm²)}}
\]

The results are presented as mean weight in grams required to break open the wound of both the sides ± SEM.

**Histopathological study**

Rats were obtained and fixed in 10% formalin solution. Tissues were trimmed longitudinally and routinely processed. Tissue processing was done to dehydrate in ascending grade of alcohol, cleaning in xylene, and embedded in paraffin wax. Paraffin wax embedded tissue blocks were made of 3 um thickness with the rotary microtome. All the slides of skin were stained with hematoxylin and eosin (H and E) stain and Masson trichrome stain. Prepared slides were examined under microscope and photographs were taken.

**RESULTS**

The proximal analysis results *H. rosa-sinensis* L.; however, powder was done as per standard procedure [Table 2]. The percentage extractive yield of *H. rosa-sinensis* L. HA extract using Soxhlet apparatus was found to be 17.48% w/w. The preliminary phytochemical studies of HRS flower revealed the presence of alkaloids, flavonoids, tannin, glycoside, and protein in hydroalcoholic extract [Table 3]. Total phenolic content and TFC were found to be 81.83 ug/mL and 376.25 ug/mL, respectively. Optimized formulation containing 5% and 10% HA-HRS extract was prepared [Table 4], both formulations were evaluated for color, appearance, pH, and viscosity [Table 5].

**Antimicrobial Study**

The antimicrobial activity of the extract, 5% and 10% gel formulation, was studied against *S. aureus* and

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**Table 1: Treatment plan for excision/incision wound model**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Model</th>
<th>Treatment</th>
<th>No. of animals</th>
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<tbody>
<tr>
<td>1.1</td>
<td>Excision/Incision</td>
<td>Group I (Control)3</td>
<td>6</td>
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<tr>
<td></td>
<td></td>
<td>Group II (Std. Povidion-iodine ointment 5%)</td>
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<td></td>
<td></td>
<td>Group III (HA-HRS 5% Gel)</td>
<td>66</td>
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<tr>
<td></td>
<td></td>
<td>Group (HA-HRS 10% Gel)</td>
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Zone of inhibition was found to be 14.24 ± 0.24 for HA-HRS extract, 12.24 ± 0.25 for HA-HRS 5% gel, and 12.36 ± 0.24 for HA-HRS 10% gel which was comparable to synthetic antibacterial standard (14.76 ± 0.005) against *S. aureus* culture. Whereas, zone of inhibition of extract and gel formulation against *E. coli* was similar to that of standard [Table 6 and Figure 1].

**Wound Healing**

No reactions were observed after 7 days of irritation study for both the formulations. Increase in wound healing was observed in animal treated with HA-HRS flower extract. In excision wound model, the wound was found to decrease significantly in Group IV (rats treated with gel containing 10% of HA-HRS extract) as compared to other (*P* < 0.0001). Wound area on the 14th day for Group III animals was 17.78 ± 3.04 and control was 67.23 ± 2.84, whereas almost complete healing was observed in Group IV treated rats and standard in Group II [Table 7 and Figure 2]. In incision study, 5% and 10% extract containing gel showed
significant (196 ± 2.5 and 220 ± 12.9) breaking strength as compared to control (144 ± 14.7) [Table 8]. Histopathological observation in incision model stained with H and E stain showed fibroblast proliferation in dermis and increased epidermal thickness for animals treated with 10% of extract containing gel. For excision model, animals treated with 10% of extract containing gel showed normal skin histology with dermis, epidermis, and hair follicle. Masson trichrome staining examination revealed moderate increase in collagen content at wound increase in collagen count at wound area for both the formulations in both the models [Figure 3].

**DISCUSSION**

The proximal analysis of *H. rosa-sinensis* L. powder was done as per standard procedure and was found within acceptance limit. The percentage extractive yield of
H. rosa-sinensis L. powder using ethanol: water (7:3) was found to be 17.48% w/w by Soxhlet extraction. Total flavonoid and phenolic content was found to be 376.25 ug/mL and 81.83 ug/mL. Extraction was evaluated for the presence of secondary metabolite by preliminary wet test which showed the presence of alkaloids, flavonoids, glycoside, tannin, and phenols in the extract. The antimicrobial activity of the HA-HRS extract showed comparatively with standard (14.76 ± 0.005) with using S. aureus culture and E. coli. HPTLC method was used as a tool for standardization of extract using quercetin as standard. Mobile phase consisting of toluene: ethyl acetate: formic acid (5:4:0.2 v/v/v) gave good and reproducible peak at Rf = 0.55 for quercetin standard and same was observed in extract. Optimized formulation containing 5% and 10% HA-HRS extract was prepared using Carbopol-940 as gelling agent. The optimized gel formulation was evaluated for color appearance, pH, spreadability, extrudability, and viscosity. The pH of HA-HRS gel was within acceptable limit 6.97 ± 0.02 for 5% HA-HRS gel and 7.02 ± 0.02 for 10% HA-HRS gel. Extrudability was measured for both formulations and was excellent (>90%). Viscosity and percentage torque value of both the formulations were found to be acceptable limits. Prepared formulation proved to be good wound healers in in vivo experimentation on rats. 10% HRS gel formulation healed wound on the 14th day of treatment in excision model. Normal histology of skin with increased epithelial layer and produced hair follicle was also observed in excision wound model for 10% HRS gel. Whereas, incision model animals showed increased epidermal thickened treated with 10% HRS gel. Based on the findings, 10% HRS gel can be good alternative to synthetic formulation.

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