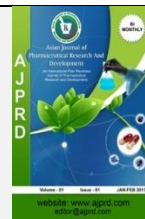


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Research Article

## Potential Emphasis of Formulated Herbal Cream on Wound Healing

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### ABSTRACT

**Aim:** The present study was to formulate and evaluate the herbal cream comprising extracts of different medicinal plants.

**Experimental:** The aqueous extract of all selected plants (*Chenopodium album*, *Coccinia indica*, *Momordica dioica*, *Praecitrullus fistulosus* and *Trichosanthes dioica*) were prepared by successive solvent extraction using soxhlet apparatus. Topical herbal cream was formulated and examined on different parameters like colour, consistency, pH, viscosity, spreadability and stability. Pharmacological evaluation of formulated herbal cream was done with excision and incision wound models.

**Results and Conclusion:** Based on the resultant findings obtained from the different evaluation parameters, it can be concluded that prepared herbal formulation was stable and safe to use in wound healing activity.

**Keywords:** Excision, Incision, Evaluation, Herbal Cream

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### INTRODUCTION

Wounds are defined as physical, chemical or thermal injuries that outcome in an opening or breaking in the reliability of the skin or the interruption of anatomical and functional integrity of living tissues.<sup>1</sup> Wound healing is the process of renovate that follows injury to the skin and other soft tissues.<sup>2</sup> The effectual management of wounds will reduce the number of complications and allow speedy return to normal function. Therefore, the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences.<sup>3</sup> Attention should be aimed at towards discovering an agent, which will accelerate wound healing. The use of medicinal plants in the management of acute and chronic wounds is common in most conventional medicine practices in the world. Herbal medicines are widely used all over the world. However, there is still a huge gap between best scientific evidence and what people actually use to treat a disease. The lack of acceptance of herbal medicines by medical community is based both on false and true grounds. It is important that study of herbal medicine is offered to all health care professionals.<sup>4</sup> The use of natural products brings a challenge to their expansion as drugs. Indeed, important medicines were developed and marketed in the past as complexes because it was not possible to purify the individual components at a sufficient scale.<sup>5</sup> Clearly, many technologies required for

the successful discovery, development and production of herbal medicines are not yet in place and efforts required for their emergence need to be substantial.<sup>6</sup> Nevertheless, while new technology is not in place, ethnopharmacology still is more efficient to discover new drugs from plants. Medicinal plants *Chenopodium album*, *Coccinia indica*, *Momordica dioica*, *Praecitrullus fistulosus* and *Trichosanthes dioica* were used in the formulation of herbal cream for the assessment of wound healing activity. Hence the present work was designed to formulate and evaluate an herbal cream containing different plant extracts for potent healing activity in various wound models.

### MATERIAL AND METHODS

#### Collection of plant materials

All the selected plants (*Chenopodium album*, *Coccinia indica*, *Momordica dioica*, *Praecitrullus fistulosus* and *Trichosanthes dioica*) leaves were collected from available sources. The leaves were cleaned, sorted and air dried under shade at room temperature. The dried leaves were converted into powder and kept in an air tight polyethylene bag until needed for the extraction process.

### Preparation of plant extracts

The powdered materials of all the plants (*Chenopodium album*, *Coccinia indica*, *Momordica dioica*, *Praecitrullus fistulosus* and *Trichosanthes dioica*) were extracted successively with water solvent to obtain aqueous extract of all plants by using Soxhlet apparatus. Obtained extracts were dried by removing the solvent under vacuum. The dried extract of each plant was weighed and stored for further use.

### Formulation of herbal cream

Ingredient of oil phase (A) was melted in a beaker by using water bath on stable stirring. Components of aqueous phase (B) were mixed together and warmed to about same temperature of oil phase (up to 70°C). The preservative methyl paraban and propyl paraban were added into aqueous phase and heated. Then oil phase was added to water phase little by little on stable stirring. Aqueous extracts of each plant were mixed with uniform stirring in the formulated cream base. Water was added finally and mixed.<sup>7-8</sup> The composition of formulated herbal cream is given in table 1.

**Table 1:** Composition of formulated herbal cream

	Ingredients	Qty.% w/w
Oil Phase	Stearic acid	1%
	White Beeswax	5%
	Cetyl alcohol	3%
Aqueous Phase	Propylene glycol	5%
	Glycerine	4%
	Methyl Paraban	0.5%
	Propyl Paraban	0.5%
	Water	Upto 100%
	Plant Extracts	<i>Chenopodium album</i>
<i>Coccinia indica</i>		5%
<i>Momordica dioica</i>		5%
<i>Praecitrullus fistulosus</i>		5%

### Evaluation of formulated herbal cream

The formulated herbal cream was evaluated in terms of various evaluation parameters.<sup>9-11</sup>

#### Organoleptic evaluation

The formulated herbal cream was evaluated for its organoleptic properties like color, odor and consistency manually.

#### Wash ability

The product was applied on hand was observed under running water.

#### pH

The pH of formulated herbal cream was determined by using digital pH meter. About 1g of the cream was weighed and dissolved in 100 ml of distilled water and its pH was measured.

### Viscosity

In the current study, viscosity of formulated herbal cream was determined by book field viscometer at 100 rpm using spindle No 7.

### Extrudability

The formulations were filled in collapsible tube container. The extrudability was determined in terms of weight of formulations required to extrude 0.5cm of ribbon of ointment in 10 seconds.

### Spreadability

In the current investigation, sample of the formulated cream was sandwiched between 2 slides. A weight of 100gm was placed on upper slide. The weight was removed and extra formulation was scrapped off. The lower slide was fixed on board of apparatus and upper slide was fixed with non flexible string on which load was applied. Time taken by upper slide to slip off was noted down. Spreadability=  $m \times l / t$

$m$  = weight tied to the upper slide,  $l$  = length of glass slide,  $t$  = time taken in seconds.

### Test for thermal stability

The formulated cream was inserting into glass bottle with the help of spatula, and taped to settle to the bottom. Filled up to two-third capacity of bottle and insert plug and tighten the cap. Filled bottle was kept erect inside the incubator at  $4 \pm 1^\circ$  for 48 hrs. The sample passed the test, if on removal from the incubator shows no oil separation or any other phase separation.

### Test for microbial growth in formulated creams

The formulated creams were inoculated on the plates of agar media by streak plate method and a control was prepared by omitting the cream. The plates were placed in to the incubator and are incubated at 37°C for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

### Pharmacological evaluation of formulated herbal cream

#### Experimental animals and Treatment protocol

Albino wistar rats weighing 100-150 g were used. The animals were maintained in well ventilated animal room for a period of 7 days prior to the experimental period. They were kept on standard pellet diet and water ad libitum. Institutional animal ethical committee permitted the study. Animals of either sex were divided into three groups for each excision and incision wound model. The grouping of animals was shown in table 2.

**Table 2:** Grouping of Animals

Group I	Control (Cream Base)
Group II	Standard (0.1% silver sulfadiazine cream)
Group III	Herbal cream formulated with aqueous extracts of all selected plants

### Excision Wound Model

An excision wound was inflicted by cutting away a 300 mm<sup>2</sup> full thickness of skin from a predetermined shaved area. Rat's wounds were left undressed to the open

environment. The cream base, standard drug cream (0.1% Silver sulfadiazine) and formulated herbal cream were applied topically to the control group, standard group and treated group respectively till the wound was completely healed. In this healing evaluation model, wound contraction and epithelialization period was monitored. Wound contraction was measured as percent contraction in each 4 days after wound formation.<sup>12</sup>

### Incision Wound Model

All animals were anaesthetized before wound creation and two full thickness paravertebral long incisions were made through the skin at the distance of about 1 cm from midline on each side of the depilated back of rat. The both edges kept together and stitched with black silk surgical thread (no. 000) and a curved needle (no. 11) was used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, wound was left undressed. Cream base, standard cream and formulated herbal cream was applied daily up to 9 days. The sutures were removed when wounds were cured thoroughly. Tensile strength, hydroxyproline content in healed tissues was determined.<sup>13-14</sup>

### Histopathological studies

The regenerated tissue from the healing wounds was collected and placed in 10% buffered formalin for histopathological studies. Sections from the regenerated tissue were examined for epithelialization, inflammation, collagen and fibroblasts.<sup>15</sup>

### Statistical analysis

The values were calculated as mean  $\pm$  S.E.M.  $P < 0.01$  or above was considered to be significant.

## RESULTS AND DISCUSSION

Formulated herbal cream was evaluated by various standard parameters and found acceptable in all limits. The colour was found greenish brown, semisolid consistency, easily washable with good extrudability. The pH, viscosity and spreadability of formulated herbal cream was found 6.10, 5905 and 7.8 respectively. There is no microbial growth and oil separation in prepared formulation. All the findings were reported in table 3.

### Effect of formulated herbal cream on % wound contraction in excision wound model

A better healing pattern with complete wound closure was observed in 16 days by standard and formulated herbal cream treated groups while it was about more than 24 days

in control rats as shown in fig 1. The increased wound contraction in the formulated herbal cream treated group may be due to the enhanced activity of fibroblasts.

### Effect of formulated herbal cream on epithelialization period in excision wound model

The epithelialization time was measured from initial day. The epithelialization time was significantly reduced in all the groups. The time taken for complete epithelialization of the excision wound was more than 24 days in control, 10 days in standard (silver sulfadiazine 0.01%) and 11 days in formulated herbal cream treated group. Early dermal and epidermal regeneration in the treated group confirmed that formulated herbal cream had a positive effect toward cellular proliferation and epithelialization. The findings were recorded in table 4.

### Effect of formulated herbal cream on hydroxyproline content in incision wound model

Formulated herbal cream groups showed significant increase in hydroxyproline content when compared to control group ( $P < 0.01$ ). The hydroxyproline content of granulated tissue in different groups were  $18.12 \pm 0.021$  mg/g tissue (control),  $70.25 \pm 0.014$  mg/g tissue (standard),  $68.26 \pm 0.002$  mg/g tissue (formulated herbal cream of aqueous extract). Increase in hydroxyproline content showed probably the formation of new blood vessels which accelerates the process of healing. The findings were recorded in table 4.

### Effect of formulated herbal cream on tensile strength in incision wound model

Tensile strength for all the treated groups on 10<sup>th</sup>, 12<sup>th</sup>, 14<sup>th</sup> day was found to be significant ( $P < 0.01$ ) than control group. The mean tensile strength of granulated tissue in different groups was  $385.23$  g/mm<sup>2</sup> (control),  $610.15$  g/mm<sup>2</sup> (standard) and  $442.72$  (formulated herbal cream of aqueous extract). Tensile strength reflects the sub dermal organization of the collagen fibres in wound. The findings were recorded in table 4.

### Histopathological studies in excision and incision wound model

In control wister rats excision and incision type of wounds shown incomplete healing as in poor fibroblasts cells, collagen fibres and blood vesicles while increased fibroblasts cells collagenation and angiogenesis occurs in standard (silver sulfadiazine 0.01%) and formulated herbal cream treated group as shown in figure 2.

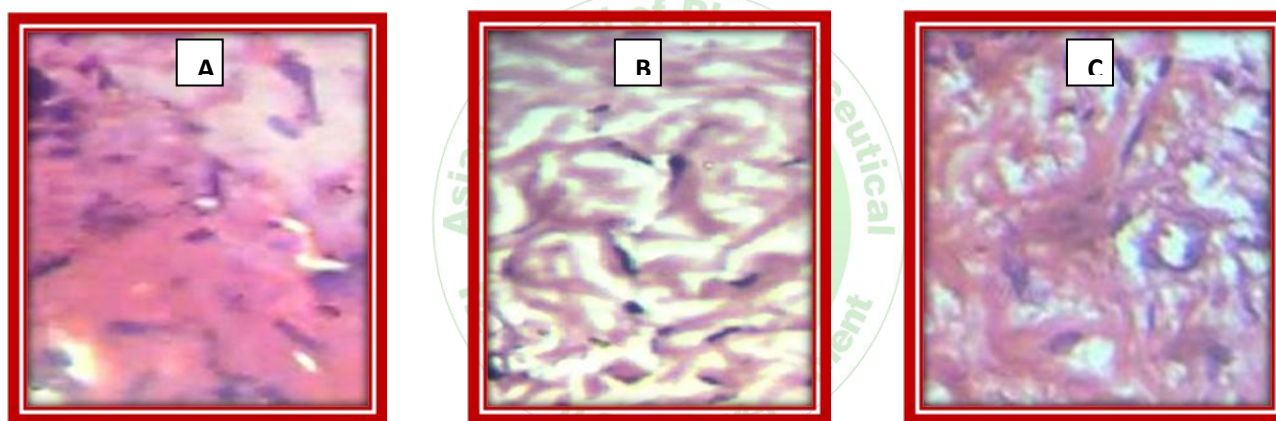
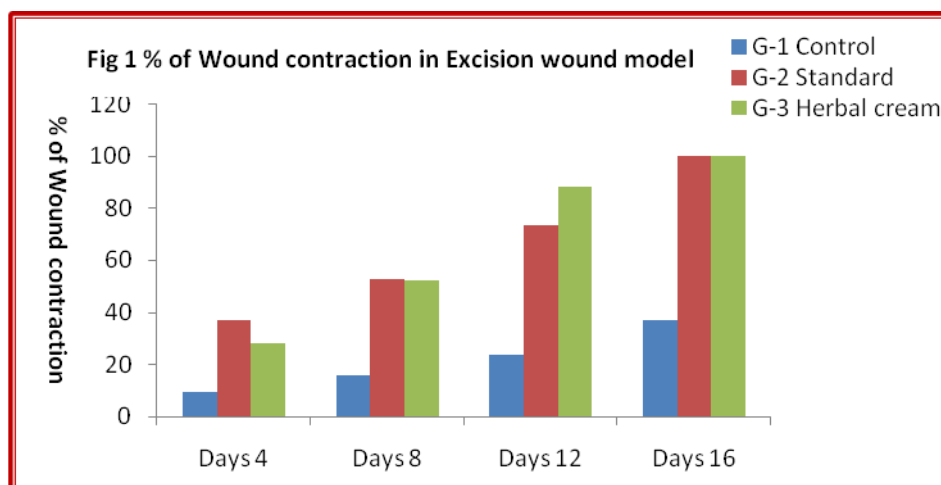
**Table 3:** Evaluation findings of formulated herbal cream

S.N.	Parameter	Findings
1	Colour	Greenish brown
2	Consistency	Semisolid
3	Wash ability	Easily
4	Extrudability	Good
5	pH	6.10
6	Viscosity	5905
7	Spreadability	7.8
8	Stability	Stable No Oil Separation
9	Microbial Growth	Absent

**Table 4:** Findings of pharmacological evaluation of formulated herbal cream

Groups	Epithelialization Period (In Days)	Hydroxyproline Content (mg/g tissue)	Tensile Strength (g/mm <sup>2</sup> )
G-I (Control)	22	18.12 ± 0.021*	385.023 ± 2.167*
G-II (Standard)	10	70.25 ± 0.014*	610.15 ± 2.717*
G-III (Herbal Cream)	11	68.26 ± 0.002*	442.72 ± 2.124*

n= 6 albino rats per group, values are represents mean± SEM \*P<0.01(comparison of I & II with III)



**Figure 2:** Histopathological characteristics of rat skin by treatment with cream base (A), Standard (B), Formulated herbal cream (C) in Excision wound model

## CONCLUSION

Plants or chemical entities derived from plants need to be identified and formulated for treatment and management of wounds. In this direction, a number of herbal products are being investigated at present. Obtained findings collectively demonstrated that formulated herbal cream possesses wound healing activity and this justifies its use for treatment of wounds. The observed efficacy may possibly be attributed to the presence of different phytoconstituents found in the extracts which are known to contribute in the wound healing properties of these medicinal plants.

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