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Original Research Paper

DESIGN, FORMULATION AND EVALUATION OF A POLYHERBAL OINTMENT FOR ITS WOUND HEALING ACTIVITY

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ABSTRACT

The present study was aimed at designing, formulating and evaluating a polyherbal ointment comprising of methanolic extracts of the leaves of *Tectona grandis*, *Ficus religiosa* and *Caesalpinia pulcherrima*. The parameters evaluated for wound healing were period of contraction and tensile strength using excision and incision models. The animals were divided into groups and were treated with polyherbal formulation, standard group and one served as control group. Nitrofurazone (0.2% w/w) was used as reference standard. The period of epithelization in the excision wound mode was found to be 13.20 days, while in case of incision wound model the tensile strength was 540 when compared to the controls ,the results were extremely significant. Different parameters like pH, viscosity, spreadability and stability were evaluated. The formulation showed good spread ability, good consistency, homogeneity, there was no change in the appearance, pH, and no phase separation noticed at the end of the stability studies. There was no evidence of skin irritation. This study has revealed that the poly herbal ointment has shown the wound healing effect due to the synergistic activity of the phytoconstituents present in the extracts and may be used a potential herbal formulation for wound healing.

Keywords: *Tectona grandis*, *Ficus religiosa*, *Caesalpinia pulcherrima*, Polyherbal cream.

INTRODUCTION

Herbal therapy predominates in traditional medicine as well as in alternative medicine practiced in the developing and the developed world. The widespread interest in drugs derived from plants is because of the belief that plants are safe and dependable, and with lesser side effects. Review of literature reveals that traditional plant drugs are beneficial for several skin related problems and for wound healing.[1] A poly herbal formulation consists of more then one herb. It is known that plants have different phytoconstituents which are responsible for the various activities that are attributed to them and when a combination of plants with these constituents are combined together it may show better activity when compared to the individual extract.[2] But at the same time presence of many constituents may lead to chemical incompatibility which may result in instability. Hence it is a challenging task to formulate a stable polyherbal formulation. Thus, the main objective of the present study is to formulate and evaluate a polyherbal formulation which may be used as a potent wound healing agent .In the present study an attempt has been made to formulate and evaluate a polyherbal formulation consisting of

the extracts of *Tectona grandis*, *Ficus religiosa* and *Caesalpinia pulcherrima* for its wound healing activity. These plants have been used traditionally for treating skin diseases, wounds and inflammation; hence an ointment prepared from these plants could be an effective treatment for different types of wounds. *Ficus religiosa* belongs to the family Moraceae. It is commonly called as the pipal in Hindi and asvattha in Sanskrit. In Ayurveda the leaves are used for the treatment of ulcers and wounds. The bark is used for the treatment of various skin diseases, scabies and in ulcers. The fruits and tender buds are used as laxatives. Its root bark is useful for stomatitis. Its roots are also good for gout. It has been reported that the leaves contain campestrol, sigmasterol and α & β Amyrins, tannins, amino acids, piperine, piperlongumine, dihydrolongumine and methyl piperate.[3-5] *Tectona grandis* is an important plant belonging to the family Verbinaceae. It is commonly referred to as teak. Literature reveals that the plant possesses activities like laxative and sedative, bronchitis, as diuretic and in the treatment of urinary discharge, common cold, as an analgesic and anti inflammatory, and in scabies. The various phytoconstituents isolated from this plant are Juglone, Betulin aldehyde, Lapchol.[6-9] *Caesalpinia pulcherrima* belongs to the family Leguminosae. Plants belonging to this species exhibit analgesic, anthelmintic, anti-ulcer, antibacterial, anti-inflammatory, antipyretic and antioxidant activities.[10-13] Hence, an effort has been made to establish the scientific validity and to investigate the possible wound healing activity of the formulated ointments made from the methanolic extracts of the above three herbs.

MATERIALS AND METHODS

Collection of Plants

The plants were collected from the Botanical Garden of Krupanidhi College of pharmacy, dried, pulverized and stored until further use.

Chemicals and Reagents

Emulsifying wax, white soft paraffin, liquid paraffin, methanol, agar which were obtained from SD Fine Chemicals, 315 - 317, T V Industrial Estate, 248, Worli Road, Mumbai, Maharashtra-400030.

Preparation of the Extracts

The extracts were prepared by macerating the powders of the leaves in methanol for 48 Hrs.

Formulation of Ointment

The required quantity of the chemicals was weighed and the polyherbal ointment was formulated by fusion method using emulsifying ointment base.

Evaluation of the Polyherbal Formulation

The polyherbal formulation was evaluated by the following physicochemical parameters[14]:

Colour and odour

Color and odor was examined by visual examination.

Loss on drying

Loss on drying was determined by placing the ointment in a petridish on a water bath and dried until constant weight was obtained.

pH

The pH of the formulation was recorded using a digital pH meter. Weighed quantity of the sample was dissolved in distilled water and stored for two hours. The measurement of pH was done in triplicate and average values were considered.

Spreadability

The spread ability was expressed in terms of times in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of certain load. Spread ability was calculated by using the formula.

$S = (M.L/T)$

Where, S = Spreadability, M = Weight tied to upper slide, L = Length of glass slides and

T = Time taken to separate the slides

Diffusion study

The diffusion study was carried out by preparing agar nutrient medium of known concentration. It was poured into a petridish and allowed to set. A hole was bored at the centre of the petridish and the prepared formulation was placed in it. The time taken for the ointment to get diffused was noted.

Skin irritation study[15]

Healthy rabbits were selected and were shaved in two different areas of the dorsal side, each about 500 mm^2.The rabbit was kept in rabbit holder and the first area was kept as control, to which emulsifying ointment base was applied, the second area was treated with polyherbal ointment. After 4hrs the skin was observed and compared with the control.

Stability studies

The stability studies were carried out for the prepared Polyherbal formulation at different temperature conditions (4˚C, 27˚ C and 37˚C) for 3 months.

Wound Healing Activity Was Carried Out Using the Following Models[16]

Excision wound model

Male and female healthy Sprague dawley rats weighing approximately 250 gm were used for the study. The animals were maintained under standard conditions during the course of the experiment. The animals were anesthetized using ether. An impression was made on the dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear of the anesthetized rat. Skin was excised to full thickness to obtain a wound area of about 500 mm^2. The animals were divided into four groups and they were treated as follows: Group I: emulsifying base, Group II: standard nitrofurazone 0.2% (w/w) ointment, Group III: polyherbal formulation and Group IV: control .The ointments were applied once daily until complete healing of wound and the wound area was measured on a millimeter scale graph paper on alternate days. The percentage of wound healing was calculated. Falling of scar was taken as the endpoint for complete epithelization and the days taken for this was considered as period of epithelization.

Incision wound model

The selection of the animals was done as mentioned above. Two paravertebral straight incisions of 6 cm were made on either sides of the vertebral column. Homeostasis was achieved by blotting the wound with a cotton swab dipped in saline and the wound was closed by means of interrupted sutures at equidistance 1 cm apart. Animals were treated daily with formulations, as mentioned above under excision wound model from 0 day to 9th post-wounding day. The tensile strength in each group is determined on the 10^{th} day by continuous, constant water flow technique.

RESULTS AND DISCUSSIONS

A wound may be defined as the loss or rupture of the cellular, anatomical or functional continuity of living tissue. Healing of skin wounds is a complex process which ultimately leads to the restoration of the injured skin. The aim of wound care is to promote wound healing in the shortest time possible.[17] The process of wound healing is promoted by several natural products which have been reported and used in Ayurveda, Siddha and Unani systems of medicines.[18] These either promote direct wound repair or exhibit related properties like anti microbial, analgesic and anti inflammatory properties which are beneficial in overall wound care. Several phytoconstituents like phenolic compounds, flavonoids, saponins are known to promote wound healing process due to their anti-oxidant and anti-microbial properties.[19,20] The results of this study have shown that the poly herbal formulation has shown significant activity when compared to the control and the base. The period of epithelization in the excision wound mode was found to be 13.20 days in case of the formulation when compared to control which was 20.00 days. While in case of incision wound model the tensile strength was 540 in case of formulation which is extremely significant(**P<0.001). The formulation was evaluated for its physicochemical properties and was subjected to stability studies. These physical parameters were within the acceptable range and the formulation was found to be stable at the end of the stability studies i.e. 90 days. The extracts of these plants contain flavonoids and phenolic compounds. The activity of the polyherbal formulation can be attributed to the presence of these constituents.

CONCLUSION

The results obtained in this study show that the combination of *Tectona grandis*, *Ficus religiosa*

and *Caesalpinia pulcherrima* formulated as polyherbal ointment accelerates the healing process by enhancing collagen formation and increasing the breaking strength of the healed wounds. This potent activity can be attributed to the phyto-constituents present in the plants which may be acting synergistically to enhance the wound healing effect.

Table1: Phytochemical Screening of the methanolic extract of *Tectona Grandis, Caesalpinia pulcherrima, Ficus religiosa*

Constituents	*Tectona Grandis*	*Caesalpinia pulcherrima*	*Ficus religiosa*
Carbohydrates	+	+	+
Proteins	+	+	+
Glycosides	+	+	+
Flavonoid	+	+	+
Tannins	+	+	+

Table 2: Physiochemical properties of the formulation

Physicochemical parameters	Formulation
Colour	Dark green
Odour	Characteristic
Loss on Drying	11.5%
p^H	6.5
Spreadability(Seconds)	15
Diffusion study	0.8cm
Skin irritation study	No skin irritation was observed
Storage(4^0,24^0,37^0 C)	Stable

Table 3: Effect of the polyherbal formulation in excision wound model

Treatment	50% wound contraction in days	Period of epithelization in days
Control	11.8±0.04	20.00±0.48
Standard	7.8±0.00	15.00±0.40*
Base	8.0±0.04	17.00±0.00
Formulation	7.00±0.2	13.20±0.02**

All values are mean SEM ±n=6, *P<0.05 indicates significant and**P<0.001 indicates extremely significant compared to the control.

Table 4: Effect of the polyherbal formulation in excision wound model

Treatment	Tensile strength
Control	300±9.25
Standard	446±12.55
Base	365±16.00
Formulation	540±9.6**

All values are mean SEM ±n=6, **P<0.001 indicates extremely significant compared to the control.

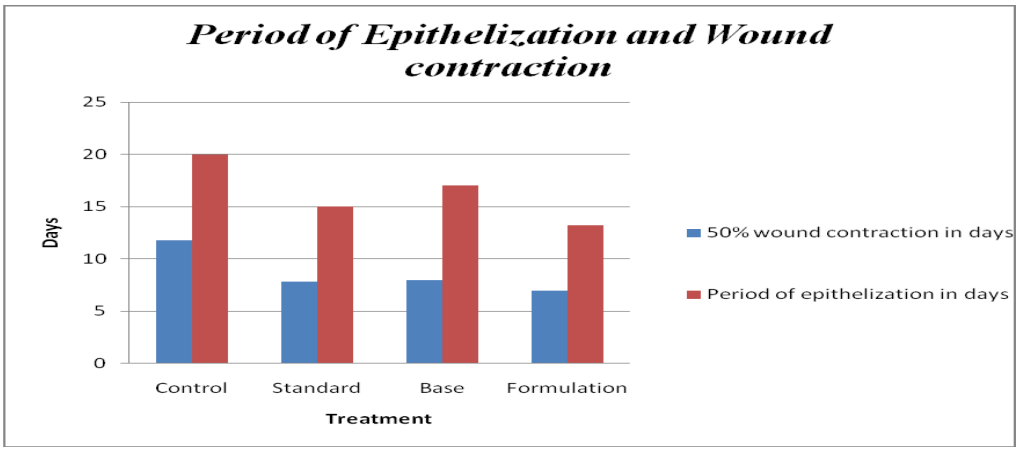

Figure 1: Effect of the polyherbal formulation in excision wound model

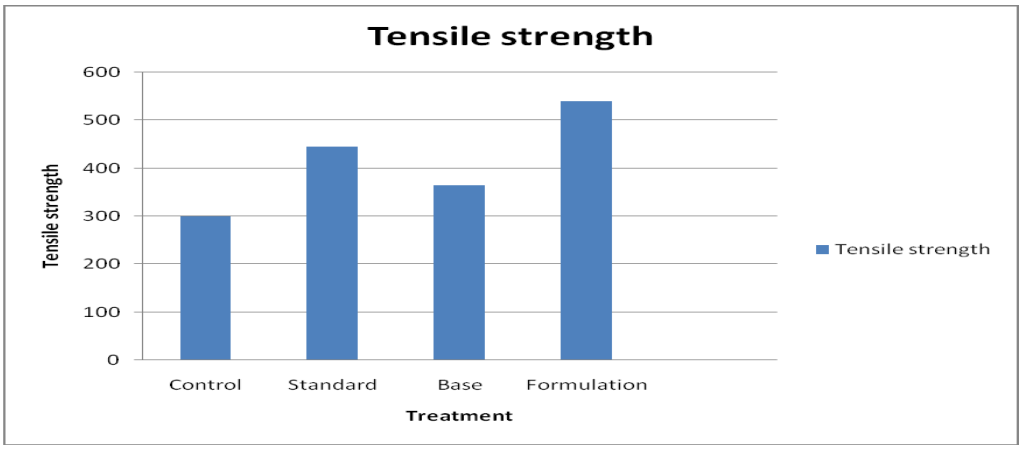

Figure 2: Effect of the polyherbal formulation in incision wound model

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