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## Formulation and Evaluation of Wound Remodeling Potential of Dual Herbal Ointment

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### ABSTRACT

The present study was aimed at formulating and evaluating a herbal ointment comprising of Ethanolic extracts of the parts of *morindacitrifolia*, and *rubiaccordifolia*. 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 herbal 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 herbal ointment has shown the wound healing effect due to the synergistic activity of the phytoconstituents present in the extracts and may be used as potential herbal formulation for wound healing.

**Keywords:** *morindacitrifolia*, *rubiaccordifolia*, Ethanolic extract, wound healing.

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## INTRODUCTION

Wound means damage, usually restricted to those caused by physical means with disruption of normal continuity of structures of external surfaces of the body, called also as injury or trauma. Wound healing is a process to restore to a state of soundness, the process of returning to health; the restoration of structure, functions of injured or diseased tissues. The healing process includes blood clotting, tissue mending, scarring, and bone healing<sup>1,2</sup>.

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<sup>3</sup>. An herbal formulation consists of two herbs. 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<sup>7</sup>. 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 dual herbal formulation. Thus, the main objective of the present study is to formulate and evaluate a dual herbal formulation which may be used as a potent wound healing agent<sup>9</sup>.

In the present study an attempt has been made to formulate and evaluate a dual herbal formulation consisting of the extracts of *morindacitrifolia* and *Rubiaccordifolia* for its wound healing activity. These plants have been used traditionally for treating skin diseases, wounds and inflammation; hence an ointment prepared from these two combination plants could be an effective treatment for different types of wounds<sup>13</sup>.

*Morindacitrifolia* (rubiaceae), also known as noni or Indian mulberry, is a small ever green plant; it has been heavily promoted for a wide range of uses; including arthritis, bladder infection, boils, burns, cancers, circulatory weakness, cold sores, eye inflammation, fractures, gastric ulcers, skin inflammation and wounds. A significant increase in the wound healing activity was observed in the animal treatment with the Ethanolic extract of *morindacalifolia*, the treated animals showed a more rapid decrease in wound size and decreased time to epithelialization compared with the controlled rats which receive plain water<sup>4</sup>.

*Rubiaccordifolia* (rubiaceae) also known as manjistha, Indian madder, distributed throughout India. The roots of this plant are of high medicinal value and are recognized as official *rubia cordifolia*, it has a variety of uses such as blood purifier, immunomodulator, anti-inflammatory,

and antioxidant. Ethanolic extract showed the presence of anthraquinone glycosides, saponins, tannins and phytosterols which may be responsible for wound healing action<sup>7</sup>.

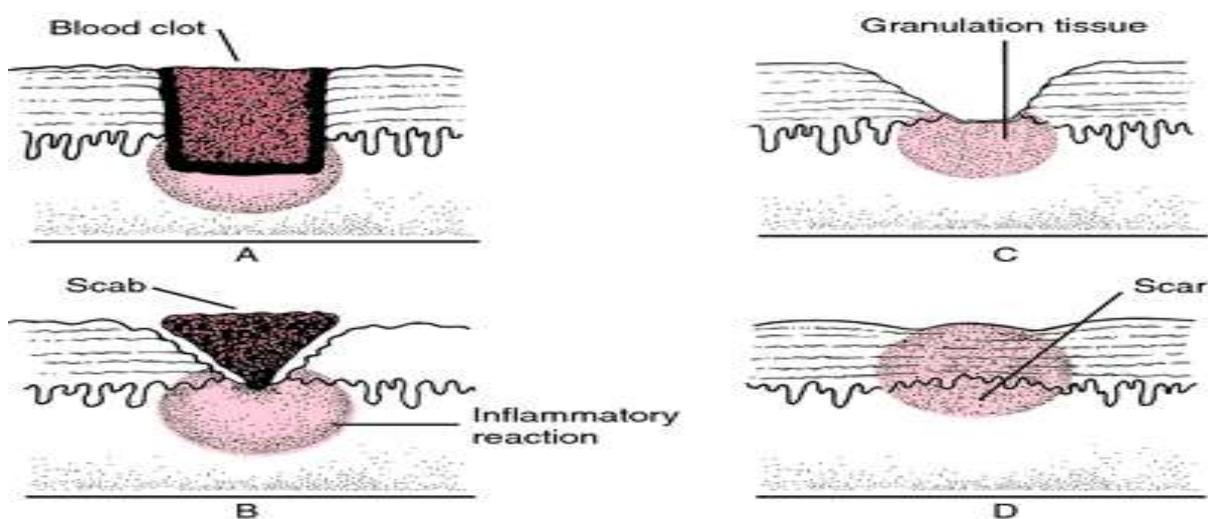
Hence, an effort has been made to establish the scientific validity and to investigate the possible Wound healing activity of the formulated ointments made from the Ethanolic extracts of the above two herbs<sup>10</sup>.

### Mechanism of Wound Healing

The response to injury, either surgically or traumatically induced, is immediate and the damaged tissue or wound then passes through three phases in order to affect a final repair:

- > The inflammatory phase
- > The fibroplastic phase
- > The remodeling phase

The inflammatory phase prepares the area for healing and immobilizes the wound by causing it to swell and become painful, so that movement becomes restricted. The fibroplastic phase rebuilds the structure, and then the remodeling phase provides the final form<sup>2,11</sup>



**Figure 1 Shows stages of wound healing<sup>14</sup>**

**Figure-A:** Incised wound is held together by a blood clot, an inflammatory process begins in adjacent tissue at the movement of injury.

**Figure -B:** After several days, granulation tissue forms as a result of migration of fibroblasts to the area of injury and formation of new capillaries. Epithelial cells at wound margin migrates to clot and seal the wound, regenerating epithelium covers the wound

**Figure-C:** Scaring occurs as granulation tissue matures and injured tissue is replaced with connective tissue.

**Figure-D:** Surrounding skin moves towards centre of wound in an effort to close the defects.

**Table 1: Some medicinal plants which shows wound healing properties<sup>6</sup>**

Sr No	Botanical name	Family	Plant/Part used
1	Rubia cordifolia linn	Rubiaceae	Roots
2	Ocimum kilimandscharicum	Laminaceae	All parts
3	Tephrosia purpuria linn	Leguminoseae	Various parts
4	Aloe vera linn	Asphodelaceae/liliaceae	Leaf
5	Barleriaprionitis L	Acatheceae	Leaf
6	Cassia alata L	Caesalpinae	Leaf
7	Kigeliapinnata sausage	Bignoniaceae	Bark
8	Sphaeranthus indicus linn	Asteraceae	Whole plant
9	Alliumcepa linn	Liliaceae	Bulb
10	Morinda citrifolia linn	Rubiaceae (noni)	Whole plant

## MATERIAL AND METHODS

### Collection of plants<sup>1,4,9,11,13</sup>

The plants were collected from the Botanical garden of Al-Ameen College of pharmacy, Bangalore, Karnataka, dried, pulverized and stored until further use.

### Chemicals and reagents:

Emulsifying wax, white soft paraffin, liquid paraffin, methanol, agar which were obtained from Institution.

### Preparation of the extracts:

The extracts were prepared by macerating the powders of the plants in ethanol for 48 Hrs.

### Formulation of ointment:

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

### Evaluation of the dual herbal formulation:

The dual herbal formulation was evaluated by the following physicochemical parameters:

#### Color and odor:

Color and odor was examined by visual examination.

#### Loss on drying:

Loss on drying was determined by placing the ointment in a Petridis 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.

#### Spread ability

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 Petridis 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:**

Healthy rabbits were selected and were shaved in two different areas of the dorsal side, each about 500mm. 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 dual herbal ointment. After 4hrs the skin was observed and compared with the control.

#### **Stability studies:**

The stability studies were carried out for the prepared dual herbal 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<sup>5,13</sup>**

##### **Excision wound model:**

Male and female healthy Sprague dawley rats weighing approximately 250gms 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<sup>1</sup>. 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: dual herbal 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 Para vertebral 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 10th day by continuous, constant water flow technique.

### **RESULTS AND DISCUSSION:**

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<sup>2</sup>. 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. 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<sup>13</sup>. The results of this study have shown that the dual 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 dual herbal formulation can be attributed to the presence of these constituents<sup>1,4,13</sup>.

**Table 2: Phytochemical Screening of the Ethanolic extract of morinda citrifolia and rubia cordifolia**

<b>Constituents</b>	<b><i>Morinda citrifolia</i></b>	<b><i>Rubiacordifolia</i></b>
Carbohydrates	+	+
Proteins	+	+

Glycosides	+	+
Flavonoid	+	+
Tannins	+	+

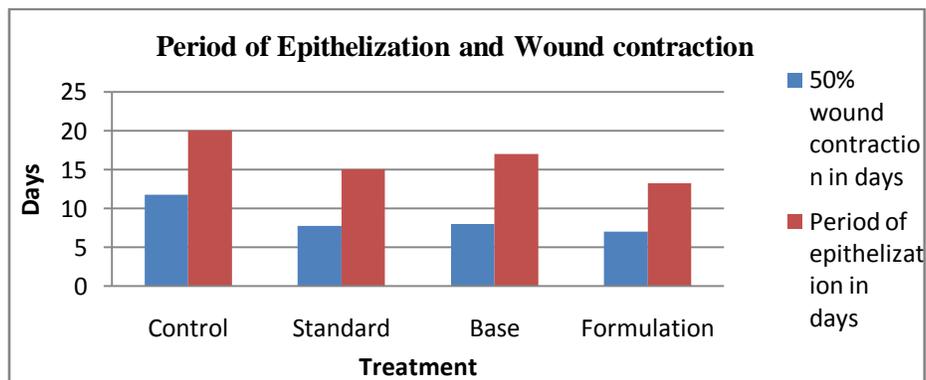
**Table 3: Physicochemical properties of the formulation:**

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

**Table 4: Effect of the dual herbal formulation in excision wound model**

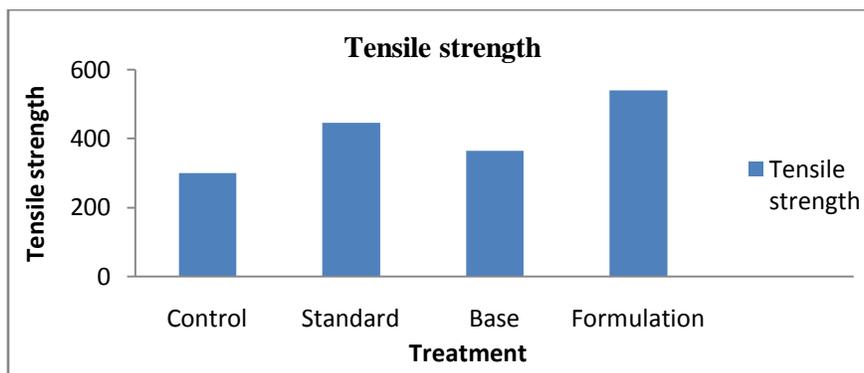
Treatment	50% wound contraction in days	Period of epithelization in days
Control	11.8±0.04	20.00±0.48
Standard	7.8±0.00	15.00±0.40*
Base	8.0±0.04	17.00±0.00
Formulation	7.00±0.2	13.20±0.02**

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

**Figure 2: Effect of the dual herbal formulation in excision wound model****Table 5: Effect of the dual herbal formulation in excision wound model**

SI No	Treatment	Tensile strength
01	Control	300±9.25
02	Standard	446±12.55
03	Base	365±16.00
04	Formulation	540±9.6**

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



**Figure 3: Effect of the dual herbal formulation in incision wound model**

## CONCLUSION:

Wound healing is a biological process that starts with trauma and ends with scar formation. The present work clearly revealed that nature provides huge number of plants that show significant wound healing activities. The results obtained in this study shows that the selected combination of *Morindacitrifolia* and *Rubiaccordifolia* formulated as dual herbal 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 phytoconstituents present in the plants which may be acting synergistically to enhance the wound healing effect.

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