



Evaluation of Polyherbal formulation for wound healing activity

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Abstract

Curcuma longa, *Tridax procumbens* and *Eclipta alba* are reported to possess wound healing, anti-inflammatory and anti-bacterial activities. The ointment formulations containing extracts of the above mentioned herbs formulated and their wound healing activity was evaluated on the experimentally induced open wound in albino rats. Formulations containing different concentration of herbal extracts were prepared and applied topically and their results were studied on the 4th day, 6th, 8th, 12th and 16th day post-operatively and compared with the controls. It was found that treated wound showed the faster rate of wound contraction than the control and wound contractions increase with the concentration of herbal extracts.

Key Words: *Curcuma longa*, *Tridax procumbens*, *Eclipta alba*, polyherbal formulation, Wound healing.

Introduction

Wounds are inescapable events in life. Wounds may arise due to physical, chemical or microbial agents. Healing is survival mechanism and represents an attempt to maintain normal anatomical structure and function. Wound healing is a process by which tissue regeneration occurs. It is complex, dynamic process of restoring integrity and tissue layer, which involves an array of inter-related and concomitant events. The process of wound repair differs little from one kind of tissue to another and is generally independent of the form of injury. Although the different steps in the wound healing processes occur in a continuous, integrated manner, it is convenient to divide the overall process into three overlapping phases and several natural components for descriptive purposes. Deficiency of certain Vitamins, trace elements and Proteins lead to delayed wound healing. Many Cytotoxics, Immunosuppressant and non-Steroidal anti-inflammatory drugs suppress wound healing. The management of wound healing is a complicated and expensive programme. Many herbs have proved to possess significant prohealing properties in different types of wounds. In this review we have made an attempt to give an insight into the different herbs having potential wound healing properties which could be beneficial in therapeutic practice. The vast literature on wound healing is focused mainly on skin, which is the

most susceptible organ in the body that interacts with the environment and, therefore, receives constant insult and damage.

However 'Mother nature has been kind to us by creating various herbs which assist in the healing process. This treatment provides fibrogenetic and concentration of collagen resulting in faster healing. The drugs selected for this work *Eclipta alba* [2, 3], *Curcuma longa*[8], *Tridax procumbens*[5-8]. These three important herbs are reported to have significant anti-bacterial, anti-inflammatory activities which are complimentary to wound healing process. The combination is used in order to enhance the wound healing activity [1-8].

Materials and Methods

The plants were selected on the basis of their anti-microbial activities and their medicinal uses reported in the literatures. The herbs were obtained from the near by villages of barabanki and were evaluated with regards to their standards specifications according to the Ayurvedic Pharmacopeia of India.

Monographic analysis of herbs

The individual herbs were evaluated with regards to their standard specifications according to Ayurvedic Pharmacopeia of India. The test carried out were Foreign organic matter, Extractive value, Ash value.

Table 1: Monographic analysis of herbs

Parameters	<i>Curcuma longa</i>		<i>Eclipta alba</i>		<i>*Tridax procumbens</i>
	Obtained value	Pharmacopeial limit	Obtained value	Pharmacopeial limit	Obtained value
Foreign organic matter	1.74 ±0.59	NMT 2%	1.65 ±0.57	NMT 2%	1.24 ±0.069
Total ash	7.34 ±0.32	NMT 9%	16.79 ± 0.78	NMT 22%	12.5 ± 0.15
Acid-insoluble ash	0.982 ±0.0117	NMT 1%	9.64±0.59	NMT 11%	0.815 ± 0.0051
Alcohol-soluble extractive	10.63 ± 0.39	NLT8%	7.05 ± 0.35	NLT5 %	10.86 ± 0.12
Water-soluble extractive	14.54 ±0.161	NLT 9%	17.35 ± 0.75	NLT15%	10.36± 0.107

* For *Tridax procumbens* pharmacopeial limits are not given

Preparation of herbal extracts

Extraction of various plant parts was done by maceration using ethanol (95% v/v). The ethanolic extract obtained were filtered and concentrated in rota-vapour at 45°C and finally air-dried.

Phyto-chemical studies

Collected extracts were subjected to various chemical tests for the preliminary determination of phytoconstituents. All extracts were mixed with equal proportion of alcohol and water (to get a hydro alcoholic sample), before subjected to various chemical reagents.

Table 2: Chemical Tests

S.No	Chemical test	<i>Curcuma longa</i>	<i>Eclipta alba</i>	<i>Tridax procumbens</i>
1	Alkaloids	-	+	+
2	Carbohydrates	-	+	-
3	Saponins	-	-	+
4	Proteins and aminoacids	-	-	-
5	Steroids	-	+	-
6	Terpenoids	+	+	+
7	Glycosides	-	+	
8	Phenolic compounds	-	-	+
9	Flavonoids	-	-	+
10	Tannins	-	-	+

* + = Present, - = Absent

Preparation of Formulation

After the preparation of herbal extract and phytochemical studies the next step was to prepare the formulation of different plant extracts. Topical formulation viz emulsifying ointment base were prepared and their composing is given in Table 3. The herbal extracts were incorporated in to the topical formulation bases in three concentrations:

- ✓ Formulation 1 contains 4% of the extract of 4% of the extract of rhizomes of *curcuma long*), 4% extract of *Eclipta alba* and 4% of *Tridax procumbens*.
- ✓ Formulation 2 contains 5% of the extract of plant 1, 5% extract *Eclipta alba* and 5% of *Tridax procumbens*.
- ✓ Formulation 3 contains 6% of extract of plant I, 6% of *Eclipta alba* and 6% of *Tridax procumbens*. The prepared formulations were then evaluated by various parameters. E.g. consistency, stability etc.

Table 3 Preparation of formulation base**Emulsifying ointment base**

Ingredients	Quantity (g)
PEG 4000	300
PEG 600	700

Table 4: Preparation of Formulations

Ingredients	Emulsifying ointment (Test I)	Emulsifying ointment (Test II)	Emulsifying ointment (Test III)
<i>Curcuma longa</i>	4%	5%	6%
<i>Eclipta alba</i>	4%	5%	6%
<i>Tridax procumbens</i>	4%	5%	6%
Emulsifying ointment base (q.s)	q.s	q.s	q.s

Wound healing activity

An excision wound model was used for studying wound healing activity. Albino rats of both sexes weighing between 150-200g were randomly divided in to 4 groups of six animals each. The back of each animal was shaved and prepared after washing with spirit. An area of about 2 cm was defined with marker on the shaven back of the animals. The circular marked area was excised with its full thickness using a surgical sterile blade and scissors under phenobarbitone anaesthesia. The area of wound was measured in sq.mm. All the samples example Control, Standard, Test I, Test II and Test III were applied once daily for the 16 days starting from the day of wounding. The observations of percentage wound closure were made on 4th, 8th, 12th and 16th post wounding days. The wound area of each animal was measured at the intervals of 24-48 hrs using transparent polythene graph paper and then tracing the area of wound on it (Approx 500 sq.mm initial wound reading was observed. Contraction was calculated from the days of measurements of wound area.

The reduction in the wound size was readily calculated by the formula: Wound contraction % = (difference in the area of the wound in mm² between the initial and on a particular post-operative day) × 100/area of the wound in mm² immediately after the wound excision.

Table 5: % Wound Contraction

Formulation code	4 th day	8 th day	12 th day	16 th day
Control	21.9	36.62	65.2	75.3
Standard	47.5	66.3	93.1	99.9
Test 1(F1)	40.9	44.8	61.3	80.2
Test 2 (F2)	45.9	49.9	65.8	82.9
Test 3(F3)	47.1	51.8	67.7	86.7

ANOVA Result - Fcal=16.4 and Ftab= 3.24

Results and Discussion

The monographic analysis of the herbs was performed according to the Indian Pharmacopeias and Ayurvedic Pharmacopeias of India. The analysis reports are given along with standard values for the herbal drugs as specified in the Ayurvedic Pharmacopeia. It was found that all the values were within the pharmacopeias limits.

In all formulations there was no considerable change in the characters like colour, odour and consistency during the course of study. The result of the wound contraction studies indicate that all formulations enhance wound healing in open wounds.

As the calculated value ($F_{cal}=16.4$) is greater than the table value ($F_{tab}= 3.24$) means there is significant difference between the formulation of different concentration and between the different days.

From % wound contraction data (Table no.5) it is concluded that formulation 3 (Test III) produced greater wound contraction compared with the other tested formulations and wound contraction increases as the day passes.

On the basis of the result obtained in the present investigation, it is possible to conclude that the ointment of the root extract of above plants has significant wound healing activity. The better activity of polyherbal formulation may be due to the synergistic action of the plants constituents present in the formulation.

Several phytoconstituents like alkaloids, saponins are known to promote wound healing process due to their anti-oxidant and anti-microbial properties. The study reveals that the wound healing activity of polyherbal formulation may be due to the combined action of phytoconstituents like alkaloids, saponins and terpenoids present in it.

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