

RESEARCH ARTICLE

Simple Evaluation of Wound Healing Activity of Polyherbal Formulation of Roots of *Ageratum conyzoides* Linn

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ABSTRACT

The present study was aim to investigate the possible effect of *Ageratum conyzoides* root extract on wound healing and is also include a comparable study of effect of polyherbal formulation containing *Ageratum conyzoides*, *Ficus religiosa*, *Curcuma longa* and *Tamarindus indica*, on wound healing process with respect to intact plant formulation through topical route. The wound healing property of *Ageratum conyzoides* appears to be due to the presence of its active principles, which accelerates the healing process and confers breaking strength to the healed wound. Further, wound healing activity by polyherbal formulation was found to be better than ageratum treated groups in rat. It may be attributed to the synergistic action of ageratum constituent and the constituent of other plants present in the polyherbal formulation.

KEY WORDS: *Ageratum conyzoides*, Wound Healing

INTRODUCTION:

A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue.¹ Wound healing studies are mainly aim to detect various means and factor influencing healing process, so they could be either used or avoid in clinical practice to favorably alter the healing process.²

Although many indigenous tribes around the world have long suspected that this ubiquitous, annual, herbaceous plant might have medicinal wound healing properties, it has not really got the attention of orthodox medical practitioners as a potential source of a healing agent which may prove to be useful in the treatment of wounds.³

Ageratum conyzoides belonging to *Asteraceae* is a common weed found everywhere in India and commonly known as goat weed, white weed, in various parts of India. The leaves are applied to the wounds act as septic and heal them quickly. The juice of the fresh plant and extract of dried plant are used to cure allergic rhinitis and sinusitis.⁴

Ficus religiosa belonging to *Moraceae* is a large deciduous tree with few or no arial roots, often epireptic. It is found throughout India, wild as well as cultivated and commonly known sacred fig, pippal, pippalah etc. Bark, leaves, tender shoots, fruits, seeds and latex are used medicinally and are generally propagated by seeds and vegetative methods.⁵

Curcuma longa belonging to *zingiberaceae* is a tufty perennial herb about 1 m. high. Rhizomes are thick, much branched, and golden-yellow in colour. Rhizome has anti-inflammatory and cholinergic properties. Also used as a pultice for wounds to avoid their cicatrisation.⁶

Tamarindus indica belonging to *caesalpiniaceae* is a large evergreen tree up to 30m high with dark grey bark having longitudinal fissures and deep cracks. Root bark is astringent, constipating, emmenagogue and tonic. Leaves are anti fungal anti-inflammatory, anthelmatic and diuretic.⁷

MATERIALS AND METHODS:

The plants were selected on the bases of their antimicrobial activities and wide medicinal uses in the traditional literatures. The ease of availability of plant is also taken into consideration during selection. Roots of *Ageratum conyzoides* (*Ageratum*), rhizomes of *Curcuma longa* (*Turmeric*), stem-bark of *Ficus religiosa* (*Peepal*) and leaves of *Tamarindus indica* (*Tamarind*) were collected and authenticated by the College of Pharmacy IPS Academy Indore. (M. P.)

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TABLE-I: Chemical Tests

S. No.	Chemical test	<i>A. conyzoides</i>	<i>C. longa</i>	<i>F. religiosa</i>	<i>T. indica</i>
1.	Alkaloids	+	-	-	-
2.	Carbohydrates	-	-	-	-
3.	Proteins and Amino acids	-	-	-	-
4.	Steroids	-	-	-	-
5.	Terpenoids (Carotenoids)	+	+	-	-
6.	Saponins	+	-	+	-
7.	Glycosides	+	-	-	+
8.	Phenolic compounds (Flavanoids)	-	-	-	+
9.	Tannins	+	-	-	-

* + = Present, - = Absent

TABLE-II Effect of Topical Application of root extract of *A. conyzoides* and Polyherbal Formulation on Excision Wound Model

Area of wound closure (sq mm \pm S.E.M)					
Groups (n)	4 th day	8 th day	12 th day	16 th day	Epithelization period (Days)
I CONTROL	395.45 \pm 1.52 (20.91%)	318.14 \pm 0.63 (36.42%)	176.10 \pm 0.69 (64.78%)	124.30 \pm 0.60 (75.14%)	22.27 \pm 0.67
II STANDARD	262.30 \pm 0.60 (47.54%)	168.10 \pm 0.67 (66.38%)	34.20 \pm 0.65 (93.21%)	00 (100%)	14.20 \pm 0.69
III TEST-I	341.33 \pm 0.65* (31.74%)	245.81 \pm 0.68* (50.84%)	130.32 \pm 0.69* (73.94%)	45.50 \pm 0.70* (90.90%)	18.66 \pm 0.70*
IV TEST-II	299.15 \pm 0.69* (40.17%)	193.65 \pm 0.69* (61.27%)	98.94 \pm 0.67* (80.21%)	20.33 \pm 0.66* (95.94%)	16.8 \pm 0.68*

EXTRACTION:

Preparation of extract of various plant parts mentioned was done by maceration, using alcohol (Ethanol 95% v/v). Extracts after filtration were concentrated at low pressure by distillation and finally air-dried.

PHYTOCHEMICAL 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.

FORMULATION:

After preparation of extract and phytochemical studies, the next step was to formulate a polyherbal preparation. An ointment with water soluble base was of first choice due to their ease of preparation and also eases of cleaning after application.

Polyethylene Glycol (PEG) Ointment base⁸, a mixture of PEG 4000 and PEG 600 found to have sufficient consistency in ratio 3:7 respectively, thus suitable for ointment preparation with concentration of 10 % w/w of extracts.

Two formulations were prepared by Fusion method e.g. one containing Ageratum leaf extract (10% w/w) in PEG ointment base (Treated as main or TEST-I) and the other one containing all four extracts of above mentioned plants parts in equal ratios i.e. containing 2.5% w/w of

each extract, equal to total 10% w/w in PEG ointment base (Treated as polyherbal or TEST-II). The prepared formulations was then evaluated by various parameters e.g. consistency, stability etc.

WOUND HEALING ACTIVITY:

Excision wound model as described by Mukherjee P K.⁹ with some modifications, using Albino rats was selected for assessing the wound healing activity. This model was employed to study the rate of wound contraction and the time required for full epithelization of the wounds. These parameters were selected because of easy availability of Albino rat and simplicity in handling them.

SELECTION AND PROCUREMENT OF ANIMALS:

After taking permission for animal studies from Institutional Animals Ethics Committee (IACE) (Reg. No.CPCSEA/444), albino rats were procured and rats of either sex weighing 150-200 gm were selected, maintained at 24-28^oC, housed individually with free access to food and water. They were fed with standard diet and kept in well-ventilated animal house with alternate dark-light cycle of 12 hrs throughout the studies.

EXCISION WOUND MODEL:

For the excision wound studies, twenty-four albino rats were taken, divided in four groups of six each. Rats depilated by removing hairs at the dorsal thoracic region

before wounding. Rats were locally anaesthetized by xylocaine jelly (Lignocaine hydrochloride gel I.P., 2% w/v) prior to excision.

Circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats under aseptic conditions and were observed throughout the study. The areas of the wounds were measured (in sq. mm) immediately by placing a transparent polythene graph paper over the wound and then tracing the area of the wound on it (Approx. area 500 sq mm). This was taken as the initial wound area reading.

All the samples e.g. CONTROL (PEG ointment base), STANDARD (Soframycin), TEST-I (*Ageratum conyzoides* root extract), and TEST-II (Polyherbal formulation), were applied once daily for 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 intervals of 24-48 hrs using tracing paper method. The percentage of wound contraction was calculated from the days of measurements of wound area.

The wound contraction was calculated as percentage reduction in wound area with respect to initial wound area while the epithelization time was noted as the number of days after wounding required for scar to fall off leaving no raw wound behind.

STATISTICAL ANALYSIS:

The results were analyzed by one-way ANOVA and a P-value less than 0.01 was considered significant.

RESULT AND DISCUSSION:

Phytochemical investigation of different extract showed the presence of alkaloids, and tannins in *Ageratum* sample only. Also no sample shows the presence of proteins and amino acids. The details of qualitative chemical tests and phytoconstituents present in the extracts are shown in Table-I.

In all formulations there was no considerable change in characters like color, odor, and consistency and there was no phase separation observed during the course of study. Also, no patches on rat skin were observed during skin irritant test.

No mortality was noticed amongst the animals in all the treated groups. The cases of wound infection were also negligible and of mild severity in the groups.

There was noticeable homogeneity in the wound contraction observed for animals in the experimental groups compared with the control group. The end scar formed was a fine linear white scar that was visible on the flank of the animals.

The studies on excision wound healing model reveals that all the four groups showed decreased wound area from day to day. However, on 16th post wounding day, Group-I animals showed 75.14% of healing (which may be due to self immunity of the animals) where as Soframycin treated animals showed 100.00% healing. On the other hand, the *Ageratum* treated group showed 90.90% of wound healing. Also, polyherbal treated group shows 95.94% healing (Table-II). All readings are found to be statistically significant and comparable with control.

The epithelization time i.e. time at which complete scar formation occur, also suggest that both *ageratum* treated group and polyherbal treated group were found to be significant and comparable with control (Table-II).

On the basis of the results obtained in the present investigation, it is possible to conclude that the ointment of the root extract of *Ageratum conyzoides* has significant wound healing activity. The above findings justify the wound healing properties of the roots of *ageratum* as suggested in the traditional literatures was comparable with control. It may be attributed to antimicrobial and haemostatic action of *ageratum*.

The wound healing property of *Ageratum conyzoides* appears to be due to the presence of its active principles, which accelerates the healing process and confers breaking strength to the healed wound.

Further, wound healing activity by polyherbal formulation was found to be better than *ageratum* treated group. It may be attributed to the synergistic action of *ageratum* constituent and the constituent of other plants present in the polyherbal formulation.

Several phytoconstituents like alkaloids¹⁰ and saponins¹¹ are known to promote wound healing process due to their antioxidant and antimicrobial activities. The study reveals that both *ageratum* and polyherbal treated groups possesses good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like alkaloids, saponins and terpenoids present in it¹²⁼¹⁴. Further investigations are necessary to determine the bioactive constituents present in the extracts used for studies.

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