

# Formulation and Evaluation of Herbal Ointment from Seeds of *Zanthoxylum Armatum* for Wound Healing Activity

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**Abstract:** The seeds of *Zanthoxylum armatum* belonging to the family Rutaceae are used in the folklore for the treatment of cholera, diarrhoea, expulsion of intestinal worms, antimicrobial, asthma, constipation and the fruits also used for wound healing activity. Hence the present study was chosen to evaluate its scientific validity. In the present study ethanolic extract of seeds of *Zanthoxylum armatum* were formulated as an ointment and screened for excision wound healing activity. Different ointment formulations of ethanolic extract of *Zanthoxylum armatum* (5% & 10%) were prepared using ointment base, white soft paraffin. These formulations were evaluated for the following parameters: pH, spreadability, grittiness, skin irritation study, pharmacological activity, stability. Wound healing studies of ethanolic extract revealed that *Zanthoxylum armatum* treated animals showed 100% wound contraction in 20<sup>th</sup> day which was comparable to that of standard ointment (povidone iodine 5%). The formulation 10% w/w was found to be more promising as it shows better physical characteristics, higher pharmacological activity and better stability compared to other formulations. So it was concluded that the *Zanthoxylum armatum* ethanolic extract shows significant improvement in excision wound contraction and hence this is a promising candidate in excision wound healing.

**Keywords:** *Zanthoxylum armatum*, Ointment formulation, Wound healing activity.

## INTRODUCTION

The use of herbal medicine for the treatment of diseases and infections is as old as mankind. The World Health Organization supports the use traditional medicine provided they are proven to be efficacious and safe.<sup>1</sup> *Zanthoxylum armatum* occurs in hot valleys of the sub-tropical Himalayas from Jammu to Assam and khasi hills at 600 to 1800m. It has an aromatic taste and smell.<sup>2-4</sup> The ethano medicinal importance of its seeds has been well known for a long time in Indian

medicinal system as a stomachic, carminative, disinfectant, antiseptic, and for the treatment of fever, dyspepsia, cholera and general debility.<sup>5-7</sup> The herbal "formula" consists of a selective combination of individual herbal ingredients that are formulated for a specific ailment or group of disease conditions. When herbs combine together, they become more potent and effective within the body than individual herb due to their activating or catalyzing influence over one another.<sup>8</sup> *Zanthoxylum armatum* plant is to be screened for wound healing activity. The process of wound healing consists of integrated cellular and biochemical events leading to re-establishment of structural and functional integrity with regain of strength in injured tissues. Wound healing is impaired in diabetic patients with infection or hyperglycemia. Diabetes mellitus is one of the major contributors to chronic wound healing problems. The diabetic patients with ulcer become at high risk for major complications which include infection and amputation.<sup>9</sup> The phases of normal wound healing include hemostasis, inflammation, proliferation and remodeling.<sup>10,11</sup> Wound healing requires adequate blood supply and nutrients to be supplied to the site of damage. These chemical signals are known as cytokines or growth factors. The fibroblast is the connective tissue cell responsible for collagen deposition that is needed to repair the tissue injury. Collagen is the most abundant protein in the animal kingdom, accounting for 30% of the total protein in the human body.<sup>12-15</sup>

## MATERIALS AND METHODS

### Collection and authentication of plant material

The seeds of *Zanthoxylum armatum* was collected from Nilgris district, Tamilnadu in June-2015. The plant material was authenticated by Dr.V.Chelladurai, Research officer- Botany, Central Council for Research in Ayurveda and Siddha, Tirunelveli. The seeds were shade dried, coarsely powdered and used for further studies.

## EXTRACTION

Dried seeds of *Zanthoxylum armatum* were extracted with ethanol in a Soxhlet apparatus for 18 hrs. The solvent was immediately filtered through funnel with cotton, evaporated by rotary evaporator. The residue which obtained in evaporation was dried and kept in a desiccator.

## FORMULATION AND EVALUATION OF HERBAL OINTMENT

The ethanolic extract of *Zanthoxylum armatum* was formulated into an ointment.

### Preparation of ointment<sup>16</sup>

Simple ointment BP were prepared by fusion method. The constituents of the base were placed together in a melting pan and allowed to melt at 70°C. After melting, the ingredients were stirred gently at 70°C for 5 minutes and then cooled with continuous stirring. Incorporation of 5g and 10g of ethanolic extract of *Zanthoxylum armatum* into the various bases was achieved by triturating in a ceramic mortar and pestle to obtain 100g of herbal ointments. The prepared herbal ointments were put in ointments jars, labelled and were stored at 25°C.

### Evaluation of ointment<sup>17,18</sup>

#### 1. Colour and odour

Colour and odour of the prepared ointment were visually examined.

#### 2. Measurement of pH

The pH of the different formulation was determined by using digital PH meter. One gram of ointment was dissolved in 100ml of distilled water and stored for 2 hours. The measurement of pH of each formulation was done after 2 hours.

#### 3. Spreadability

The spreadability of the ointment was determined by measuring of spreading diameter of 1g of ointment between two horizontal plates (20×20cm) after 1min. The standard weight applied on the upper plate was 125g.

#### 4. Grittiness

All the formulations were evaluated microscopically for the presence of particles if any. No appreciable particulate matter was seen under light microscope. Hence it is assumed that obviously the ointment preparation fulfils the requirement of "freedom from particular matter and from grittiness" as desired for any topical preparation.

#### 5. Skin irritation study

In skin irritation study six female Albino Wistar rats weighing between (150-200) were used. Animals were divided into 2 groups. Hairs were depleted from back of the sides and area 4cm<sup>2</sup> was marked on both the sides. One side served as control while other served as test I and II. The animals were used after 24h. The ointment were applied (500mg/kg) once a day for 7 days and site was covered with cotton bandage and observed for any sensitivity and the reaction if any graded.

#### 6. Stability studies

The stability studies were carried out for the prepared formulations were filled in to aluminum collapsible tubes and stored at room temperature 37°C ± 0.5 °C. All the formulations were stored for a period of six weeks. At the end of six weeks they were evaluated for physical parameters and integrity of the product.

## EVALUATION OF WOUND HEALING ACTIVITY

The wound healing capacity of the ointment prepared from the ethanolic extract of the seeds of *Zanthoxylum armatum* was evaluated.

### Statistical Analysis

The data is expressed as mean ± SEM and subjected to students't test and the level of significance was set at p < 0.001.

### Materials and method

#### Method

Wound healing activity was evaluated by the Excision wound model.

#### Experimental Animal

Healthy Wistar Albino rats of either sex and approximately the same age, weighing between 150-200g were used for the study. They were individually housed, maintained in clean polypropylene cages containing paddy husk bedding and fed with standard diet and water *ad libitum*. Clearance from the Institutional animal ethical committee was obtained for carrying out the study. Approval no (16/243/CPCSEA).

## EXPERIMENTAL PROCEDURE

### Excision wound model<sup>19,20</sup>

- Healthy Wistar albino rats of either sex weighing 150-200gm were used.
- Animals were housed under standard condition of temperature (23±1°), 12h light/dark cycle and fed with standard pellet diet and water *ad libitum*

- All the rats except rats of Normal control were inflicted with excision wound under anaesthesia.
- A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats.
- Wound was cleaned with cotton swab soaked in alcohol.
- The animals were divided into 5 groups, each group containing 6 animals until the wound was completely healed (approximately 20 days) the ointment was applied.

**Table 1: Experimental Procedure**

S.NO	GROUP	NAME OF THE GROUP	TREATMENT	NO.OF.ANIMALS USED
1	I	Normal control	Water	6
2	II	Control	Treated with ointment base for 20 days	6
3	III	Standard	0.5% w/w povidone iodine ointment- 20 days	6
4	IV	Test group-I	5% w/w test ointment -20 days	6
5	V	Test group-II	10% w/w test ointment-20 days	6
Total				30

**PARAMETERS STUDIED<sup>21-23</sup>**

- The **wound half closure time**  $Wc_{50}$  was calculated by litchifield and wilcoxon method.
- **Percentage wound contraction** was monitored by measuring wound area, on alternate days till the wound were completely healed. Wound contraction was calculated as percentage reduction in wound area.

- **Epithelialization time** was measured in days indicated by fall of scar leaving no raw wound behind.

**RESULTS AND DISCUSSION**

**FORMULATION AND EVALUATION OF HERBAL OINTMENT**

**Table 2: The formula used for preparing the ointment**

FORMULATION	INGREDIENTS	CONCENTRATION % W/W
SIMPLE OINTMENT(B.P) ( 100gm )	EXTRACT	10
	WOOL FAT	4.5
	CETOSTEARYL ALCOHOL	4.5
	HARD PARAFFIN	4.5
	WHITE SOFT PARAFFIN	76.5

**Table 3: Physical evaluation**

Formulation	Colour	Appearance	Grittiness	Spreading diameter (cm)	Ph
Control	White	Clear and Transparent	-	4.5	6.7
Test – I (5%)	Pale brown	Clear and Transparent	-	4.5	6.7
Test – II (10%).	Brown	Clear and Transparent	-	4.4	6.8

- No grittiness

**Table 4: Skin irritation study for the ointment**

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	A	A	A	A	A	A
Formulation- I	A	A	A	A	A	A	A
Formulation- II	A	A	A	A	A	A	A

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

**Acute dermal irritation test**

All the three animals were observed. No signs of erythema and oedema were observed during 14 days. So the test substance is found to be safe and Non irritant.

**Wound healing activity**

➤ **Excision wound model**

The results of the wound healing effects of the ointment of *Zanthoxylum armatum* are given in **Table (5-7)**.

**Wound half closure time  $Wc_{50}$  calculated by Litchfield-Wilcoxon method**

**Table 5: Litchfield-Wilcoxon method**

Drug	Wound contraction as $Wc_{50}$ (days)
Control	9.05±0.01
Standard	7.57±0.09
Formulation-I	7.75±0.11
Formulation-II	7.25±0.15*

**Percentage of wound contraction.**

\*\*  $p < 0.001$  vs respective control by students "t" test

**Table 6: percentage of wound contraction and Period of epithelization**

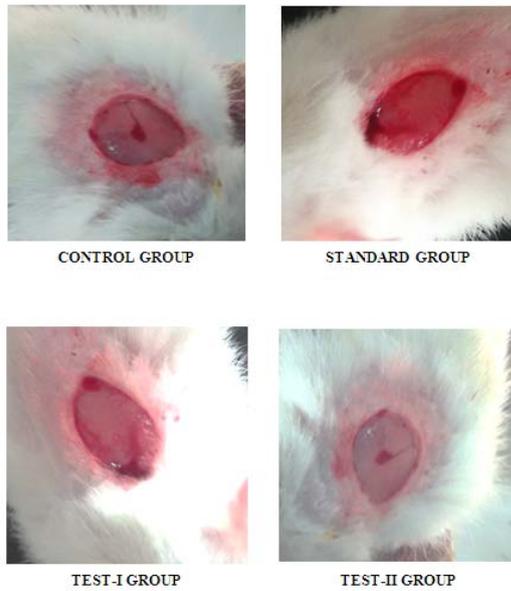
Post wounding days	Control	Standard (5% povidone iodine ointment)	Formulation-I 5% w/w	Formulation-II 10% w/w
Day 0	3±0.00	2.96±0.04	2.98±0.03	2.98±0.04
Day 4	8.75±0.81 (35.1%)	10.88±0.49 (43.5%)	10.47±0.81 (41.9%)	10.67±0.82 (43.6%)
Day 8	10.80±0.60 (42.1%)	13.42±0.67 (53.6%)	14.82±0.67 (52.2%)	15.02±0.67 (60.5%)
Day 12	15.34±1.04 (61.4%)	18.98±0.63 (75.9%)	20.35±0.81 (74.42%)	20.87±0.82 (82.43%)
Day 16	18.71±0.61 (74.8%)	23.14±0.41 (84.5%)	22.83±0.23** (83.16%)	24.01±0.63 (91.32%)
Day 20	24.24±0.04 (97.1%)	24.83±0.63 (99%)	24.98±0.67 (98.2%)	25.53±0.06 (100%)
Period of epithelization (days)	21.73±0.25	19.24±0.23	17.67±0.25	15.45±0.43

Values are mean ± S.E.M of 6 animals in each group. Number in parenthesis indicates percentage of wound contraction. \*\*  $p < 0.001$  vs respective control by students "t" test

**Table 7: Percentage wound contraction**

% Wound contraction	Control	Standard (5% povidone iodine ointment)	Formulation-I 5% w/w	Formulation-II 10% w/w
Day 4	(35.1%)	(43.5%)	(41.9%)	(43.6%)
Day 8	(42.1%)	(53.6%)	(52.2%)	(60.5%)
Day 12	(61.4%)	(75.9%)	(74.42%)	(82.43%)
Day 16	(74.8%)	(84.5%)	(83.16%)	(91.32%)
Day 20	(97.1%)	(99%)	(98.2%)	(100%)

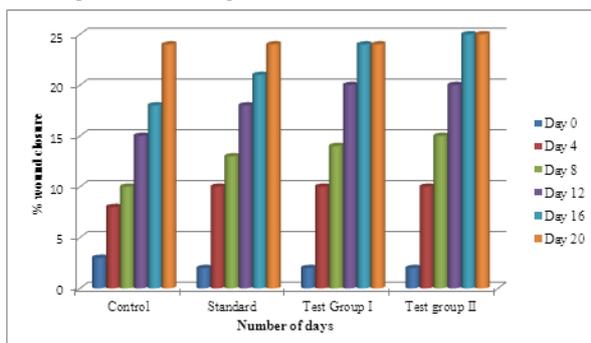
**Fig 1: INITIAL WOUND**



**Fig 2: 20<sup>th</sup> Day wound contraction**



**Fig 3: Percentage of wound contraction**



## CONCLUSION

Formulation and evaluation of ointment

- The ointment base was made with white soft paraffin and two different concentrations of the ointment was made using the ethanolic extract of seeds of *Zanthoxylum armatum* the ointment formulated were of 5% w/w and 10% w/w.
- The ointments were evaluated for various parameters such as Physical appearance, pH, grittiness and spreability. The ointment was clear and transparent with almost neutral pH. It showed no grittiness and also had a good spreability. The skin irritation studies were carried out and it was seen to be non-irritating. Showed there is no erythema was found.

Pharmacological evaluation by excision wound model:

- It was observed that the wound healing contracting ability of the extract ointment in different concentrations was significantly greater than that of control. The 10% ointment showed better activity(100%) which was comparable to that of standard(99%).
- From the results obtained, it is evident that the herbal formulation has significant wound healing activity in excision model of wound healing and hence, justifying its use in traditional practice.

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