

**PHYTOCHEMICAL SCREENING, FORMULATION AND EVALUATION OF WOUND HEALING ACTIVITY OF ROOT EXTRACT OF *ERIOLAENA HOOKERIANA* WT.ARN. OINTMENT ON RATS****K. Kishore Kumar<sup>\*1</sup>, K. Gnananath<sup>2</sup>, S. Sujatha<sup>3</sup>, B. Aswani<sup>4</sup> and J. Sivajothi<sup>5</sup>**<sup>\*1</sup>Professor, Mallareddy College of Pharmacy, Secunderabad, Telangana, Pin-500014.<sup>2</sup>Junior Research Fellow, Vishnu College of Pharmacy, Bhimavaram, A.P.<sup>3</sup>Professor, Narayana Pharmacy College, Nellore, A.P, Pin-524002.<sup>4</sup>Assistant Professor, Narayana Pharmacy College, Nellore, A.P, Pin-524002.<sup>5</sup>Assistant Professor, St ann' College of Pharmacy, Chirala, A.P.**\*Corresponding Author: K. Kishore Kumar**

Professor, Mallareddy College of Pharmacy, Secunderabad, Telangana, Pin-500014.

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**ABSTRACT**

**Objective:** Formulation and evaluation of herbal ointment of *Eriolaena hookeriana* Wt. & Arn. root extract for wound healing potential by excision and incision models on male wistar rats. **Methods:** Wound healing activity was studied in two types of model in rat's viz. excision and incision. In case of the excision wound model parameters like wound contraction and period of epithelialization were studied while in incision wound model, tensile strength of the wound was measured. **Results:** In both the models treatment of wound with ointment containing 5% (w/w) extracts showed good wound healing activity ( $p < 0.01$ ) in comparison with control. **Conclusion:** The results of present study suggested that extracts of *Eriolaena hookeriana* Wt. & Arn. Possess potent wound healing activity justifying its use in folklore.

**KEYWORDS:** *Eriolaena hookeriana*, Excision method, Incision method, Tensiometer.**1. INTRODUCTION**

In India, it is reported that traditional healers use 2500 plant species and 100 species of plants serve as regular sources of medicine. Even many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. These plant remedies both single plant and multiherbal preparations are used since ancient times even if the mechanism of action and efficacy of very few of them have been evaluated scientifically. *Eriolaena hookeriana* Wt & Arn, family *malvaceae*, a small tree, commonly found in cleared slopes in full sun at 750-1000 m central and south India.<sup>[1,2]</sup> There are about 8 to 10 species found in the world wide i.e. *E. candollei*, *E. glabrescens*, *E. hookeriana*, *E. kwangiensis*, *E. lushingtonii*, *E. quiquelocularis*, *E. spectabilis* and *E. wallichii*.

The leaf petioles are about 5 cm long. The blades are orbicular, with a diameter of around 10 cm, cordate at the base, acuminate at the apex and with serrate margins, and prominent veins on the underside. They are thinly stellate-pubescent above and rusty-tomentose below. The inflorescence is axillary. The peduncle is longer than the leaves, and bears many flowers. The bracteoles are 2–2.5 cm long and finely dissected.<sup>[3]</sup> Local name of the plant is Narabotuku and it is claimed for its use in wounds and

various infections.<sup>[2]</sup> In developing countries, a huge number of people lives in extreme poverty and morbidity are high due to lack of safe water and medicine, they have no alternative for primary health care. Therefore, the need to use medicinal plants as alternatives to orthodox medicines in the provision of primary health care cannot be over-emphasized.

Herbal medicines have received much attention as sources of lead compounds since they are considered as time tested and relatively safe for both human use and environment friendly.<sup>[4]</sup> They are also cheap, easily available and affordable. Many medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. These plant remedies (both single plant and multi-herbal preparations) are used since ancient times even if the mechanisms of action, toxicity and efficacy of very few of them have been evaluated scientifically. Wound healing is the process of repair that follows injury to the skin and other tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase protein (Collagen) production. Later, the epithelial tissue is regenerated.<sup>[5]</sup> *Eriolaena hookeriana* Wt & Arn plant is available in most of the parts of Andhra Pradesh state, no scientific study was made on this medicinal herb. Keeping this in

view, an attempt was made to explore its phytoconstituents and wound healing potential of the plant.

## 2. MATERIALS AND METHODS

### 2.1. Collection of plant material

*Eriolaena hookeriana* Wt & Arn plant was collected from Horsely Hills, near to Madanapally of Chittoor district, A.P., India, botanically identified and authenticated. The root was separated carefully, washed thoroughly, shade dried powdered by mechanical grinding. This powder was used for further studies.

### 2.2. Preparation of extracts

250 g of root powder was passed through sieve no. 60 and packed in Soxhlet apparatus and extracted using methanol and water as solvents. The filtrate was concentrated in rotary evaporator and the extracts were calculated for their yield and stored in desiccators. The extracts were designated as ALEH for methanolic and AQEH for aqueous extract respectively.

### 2.3. Phytochemical Screening

#### 2.3.1. Preliminary Phytochemical Screening

*Eriolaena hookeriana* root methanolic (ALEH) and aqueous extract (AQEH) were subjected to qualitative chemical tests to identify the nature of the phytoconstituents i.e. flavonoids, alkaloids, glycosides, steroids, saponins, terpenoids and tannins according to the standard methods.<sup>[6-8]</sup>

#### 2.3.2. Total Flavonoid content

Aluminium chloride colorimetric method<sup>[9]</sup> was used for determination of flavonoid content. 1 mL of sample was mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420 nm with ultraviolet (UV) visible spectrophotometer. The content was determined from extrapolation of calibration curve which was constructed by preparing standard Quercetin solutions (2-10 µg/mL) in methanol. The concentration of flavonoid was expressed in terms of mg/mL.

#### 2.3.3. Total phenolic content

The amount of total phenolic content of the extracts was determined by Folin-Ciocalteu<sup>[10]</sup> reagent as oxidizing agent, gallic acid as standard. Exactly 0.5 mL of the extract was transferred to a 100 mL erlenmeyer flask and the final volume was adjusted to 46 mL by addition of distilled water. 1 mL of Folin-Ciocalteu reagent was added and incubated at room temperature for 3 min. 3 mL of 2% sodium carbonate solution was added and the mixture was shaken on a shaker for 2 h at room temperature. The absorbance was measured at 760 nm. Gallic acid was used as the standard (20-100 µg/mL) for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent.

### 2.4. Preparation of ointment base

#### 2.4.1. Optimization of ointment base

In the present study water soluble base was selected after performing stability studies for different ointment bases. The selected ointment base containing Polyethylene Glycol 400 (PEG 400) and Polyethylene Glycol 4000 (PEG 4000) in the ratio of 60:40 respectively.

#### 2.4.2. Preparation of ointment

The PEG 400 and PEG 4000 were melted separately on a hot plate. Warmed the mixture to about 45°C. The mixture was removed from the hot plate and low molecular weight PEG 400 was transferred into high molecular weight PEG 4000 and stirred until congealed. To incorporate the extract into the bases pulverised extracts with small amount of base in a mortar and pestle. Wetted the extract with a levigating agent and incorporated into the ointment base. The stability studies like colour odour and pH were performed for 45 days. In this study 15gm 5% w/w ointments were prepared for each extract for studying wound healing activity.<sup>[11]</sup>

### 2.5. Pharmacological activity

#### 2.5.1. Animals used

Male wistar rats are procured from National Institute of Nutrition (NIN), Hyderabad. Animals weighing 150–180 g were selected for the experiment (n=6). The rats were used after acclimatization to the laboratory environment for a 7- day period. They were kept in the departmental animal house at 26±2°C, light and dark cycles of 10 and 14 h, respectively. Animals were provided with rodent diet (NIN) and water and *libitum*. All the experimental procedures were approved by Institutional animal ethical committee of Vaagdevi College of Pharmacy, Hanamkonda, Andhra Pradesh, India vide approval no. CPCSEA/VCOP/2011/10/3/7. In the present investigation, the rats were divided into four groups (n = 6):

Group- I was the control Group which receive ointment base,  
Group-II was treated with reference standard (0.2%, w/w Nitrofurazone ointment),  
Group-III received ALEH ointment 5% (w/w),  
Group- IV received AQEH ointment 5% (w/w).

#### 2.5.2. Excision wound model

Animals in each group were anaesthetized by open mask method with anesthetic ether. The rats were depilated on the back. One excision wound was inflicted by cutting away a 500 mm<sup>2</sup> full thickness of skin from a predetermined area. The wound was left undressed to the open environment. Then the extracts and standard were administered topically for 16 days. Contractions, which contribute for wound closure, were studied by tracing the raw wound. Wound area was measured by retracing the wound on a milli meter scale graph paper every alternate day. The degree of wound healing was calculated<sup>[12-14]</sup>, period of epithelization was also calculated and compared with that of control group.

Wound contraction was calculated as percent reduction in wound area using following formula;

$$\% \text{ of wound closure} = \frac{\text{wound area on day 0} - \text{wound area on day N}}{\text{Wound area on day 0}} \times 100$$

Where N = number of days 2<sup>nd</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup> and 16<sup>th</sup> day.

### 2.5.3. Incision wound model

Animals in each group were anaesthetized and one paravertebral-long incision was made through the skin and cutaneous muscles at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment. All the groups were treated in the same manner as that of excision wound model. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk at 0.5cm intervals; surgical thread (No. 000) and a curved needle (No. 11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed. Sample extract along with simple ointment (control) and standard drug were topically administered once daily for 9 days; when wounds were cured thoroughly the sutures were removed on the 10<sup>th</sup> day and tensile strength was measured with a local made Tensiometer.<sup>[15]</sup>

### 2.5.4. Tensile strength

The tensile strength of a wound represents the degree of wound healing. Usually wound-healing agents promote a gain in tensile strength. The sutures were removed on the 9<sup>th</sup> day after wounding and the tensile strength was measured on the 10<sup>th</sup> day.<sup>[16-18]</sup> The mean tensile strength on the two paravertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength different extracts ointment-treated wounds were compared with control groups. The tensile strength

increment indicates better wound healing stimulation by the applied drug. Tensile strength was calculated using the following formula.<sup>[19]</sup>

$$\text{Tensile strength} = \frac{\text{Breaking strength (g)}}{\text{Cross-sectional area of skin (mm}^2\text{)}}$$

### 2.5.5. Tensiometer

In the study experiment local made Tensiometer was used, which consists of a wooden board to which four nail was fixed. To one end the nail thread tied which was fixed, where as to another end easy movement of thread was allowed with the help of pulley to the edge of thread weighing balance was attached. Two clamps were tied to the thread on each side. The rats were anaesthetized individually and were placed in wooden board between nails. The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5 cm away from the wound. Analytical weights were placed on the weighing balance by increasing the weights until the healed wound opens. Thus tensile strength of wound was measured.

## 3. RESULTS

**3.1. Percentage of yield:** The percentage yield of the extracts was 4.5% w/w, 3.6% w/w for ALEH and AQEH respectively.

### 3.2. Preliminary Phytochemical Screening

Results of preliminary phytochemical screening studies on the prepared extracts of roots of *Eriolaena hookeriana* revealed the presence of chemical constituents like flavonoids, saponins, tannins, phenols and alkaloids in the methanolic and aqueous root extracts. In ALEH alkaloids, saponins and tannins are also present along with flavonoids and phenolic content. In case of AQEH tannins, saponins are present with flavonoids and phenolic compounds. Results of the phytochemical screening of *E. hookeriana* are presented in table 1.

**Table. No. 1: Preliminary Phytochemical screening of both extracts of *E. hookeriana***

S.No.	Phytoconstituents	ALEH	AQEH
1	Alkaloids	+	-
2	Carbohydrates	-	-
3	Glycosides	-	-
4	Saponins	+	+
5	Phenolic compounds	+	+
6	Tannins	+	+
7	Gums and mucilages	-	-
8	Flavonoids	+	+

‘+’ indicates present and ‘-’ indicates absent.

### 3.3. Total flavonoid content

Total flavonoid content of AQEH, ALEH (1mg) equivalent to 3.56 µg, 3.51 µg respectively of quercetin was detected.

### 3.4. Total phenolic content

In the present study total phenolics content of ALEH, AQEH (1mg) equivalent to 27.5 µg and 31.54 µg respectively of gallic acid was detected.

### 3.5. Stability studies of herbal ointments

There is no significant change in colour, pH and odour at 4°C and room temperature on 45<sup>th</sup> day of observation in

case of both 5% ALEH and AQEH herbal ointments (Table 2).

**Table. NO. 2: Stability studies of both herbal ointments of *E. hookeriana***

Ointment	Parameter	Initial Day		45 <sup>th</sup> day	
		4°C	Room Temperature	4°C	Room Temperature
5% ALEH	Colour	Brown	Brown	Brown	Brown
	Odour	Nil	Nil	Nil	Nil
	pH	6.62	6.61	6.60	6.55
5% AQEH	Colour	Black	Black	Black	Black
	Odour	Characteristic	Characteristic	Characteristic	Characteristic
	pH	6.63	6.61	6.60	6.58

### 3.6. Wound models

Effect of topical application of ointments containing 5% ALEH and AQEH extracts were determined. Results obtained from both excision and incision wound models have been expressed as mean±S.D. and were compared

(Table 3) with the corresponding control (simple ointment) values. The percentage of wound contraction was calculated as a percentage of the corresponding 0 day's (original) wound area (mm<sup>2</sup>).

**Table.No. 3: Effect of topical application of ointments containing ALEH 5% and AQEH 5% extracts of roots on wound contraction of excision wound model.**

Post wound day	Wound Area in mm <sup>2</sup> (% wound contraction)			
	Control	Standard	ALEH 5%	AQEH 5%
0	500.76±20.86(0)	517.48±24.64 (0)	497.31±20.44 (0)	500.76±20.86 (0)
2	455.60±14.24(9)	406.81±24.32 (21.3) <sup>a</sup>	428.58±15.57 (13.8)	398.06±29.47 (20.4) <sup>b</sup>
4	395.08±28.23(18.5)	216.35±19.04 (58.2) <sup>a</sup>	236.26±16.23 (52.4) <sup>a</sup>	227.18±14.38 (54.6) <sup>a</sup>
6	256.41±21.95(42.8)	133.05±14.42 (74.2) <sup>a</sup>	152.45±16.20 (69.3) <sup>a</sup>	125.55±17.28 (74.9) <sup>a</sup>
8	249.88±11.95(50.1)	77.85±14.81 (84.9) <sup>a</sup>	96.2±17.8 (80.5) <sup>a</sup>	76.65±16.65 (84.7) <sup>a</sup>
10	229.30±10.06(54.2)	43.4±6.71 (91.6) <sup>a</sup>	60.28±7.18 (87.8) <sup>a</sup>	35.4±9.34 (92.9) <sup>a</sup>
12	207.58±13.37(58.5)	14.33±3.01 (97.2) <sup>a</sup>	26.20±6.49 (94.7) <sup>a</sup>	11.81±4.34 (97.6) <sup>a</sup>
14	152.35±12.68(69.5)	5.01±2.83 (99) <sup>a</sup>	14.33±3.51 (97.1) <sup>a</sup>	4.65±2.64 (99) <sup>a</sup>
16	131.1±7.65(74.8)	2.75±1.59 (99.4) <sup>a</sup>	5.81±2.31 (99) <sup>a</sup>	1.78±1.4 (99.6) <sup>a</sup>

## 4. DISCUSSION

The effect of wound healing activity in this model was evaluated by determining the tensile strength of the incision wound of different groups viz. control treated with Simple ointment base, standard group treated with drug nitrofurazone and the test group treated with the extracts.

### 4.1. Excision model

In order to study the wound healing abilities of *E. hookeriana*, an attempt has been made by employing the topical treatment of extracts on the excised wounds. We have clearly observed an enhanced wound contraction induced by the AQEH and ALEH ointments. The area of wound was measurement on the days 2, 4, 6, 8, 10, 12, 14 and 16 days of post surgery in all the groups. The standard group, treated with 0.2% w/w Nitrofurazone ointment has shown little contraction compared with ALEH and AQEH compounds on 2<sup>nd</sup> day. A very rapid closure of the wound in the both ALEH and AQEH treated groups observed between 4 and 8 days of post surgery ( $p < 0.01$ ). After day 8 of post surgery, wound closure was gradual till the total closure of the wound. However, in the standard group, treated with

Nitrofurazone, has shown gradual closure of the wound and on the 16<sup>th</sup> day total closure of the wound observed by 5% AQEH. This could be attributed to the enhanced contractile property of myofibroblast resulting in the increase of epithelialization especial in ALEH ointment. The presence of phenolics in *E. hookeriana* also supports these results as phenolic compounds are known for free radical scavenging property.

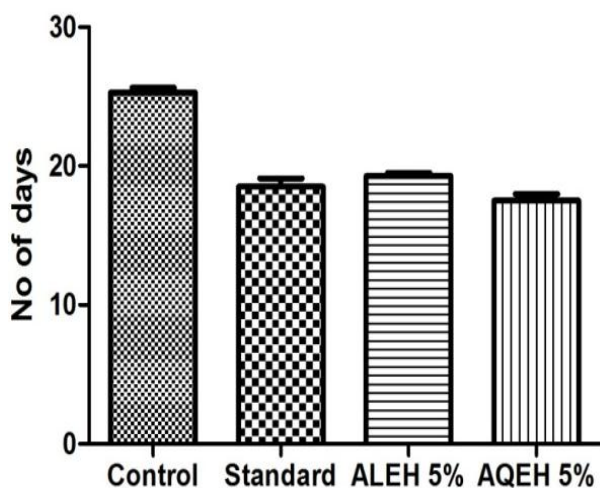
On the day 16 of post surgery, mean wound area of ALEH and AQEH and standard groups were 5.81±2.31, 1.78±1.4 and 2.75±1.59 mm<sup>2</sup>, respectively, but in control group wound area was 131.1±7.65 mm<sup>2</sup>, indicating that both extracts are showing wound-healing property comparable to that of standard group. Total wound closure was observed by the 16 day of post surgery in all the treated groups and by 21<sup>st</sup> day in control group. The period of epithelisation period was significantly ( $P < 0.001$ ) reduced from 24 days (control) to 17 days (Fig. 2). The wound healing activity due to may be presence of flavanoid and phenolic content in both alcoholic and aqueous plant extracts along with other chemical constituents (Table 1). These chemical constituents were stable for 45 days at 4°C without significant change in



colour, pH and odour in case of both 5% ALEH and AQEH herbal ointments.



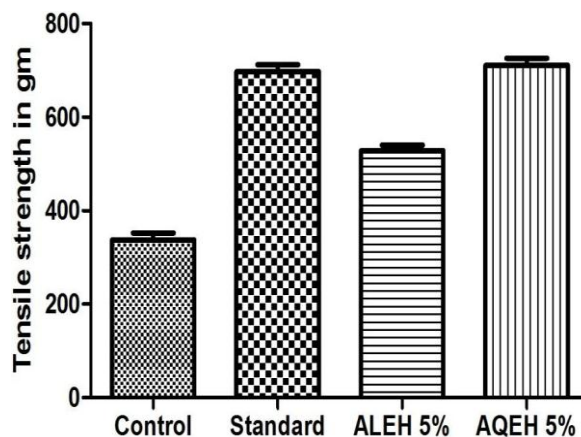
**Fig.1. Effect of Wound contraction by topical application of ointments containing 5% ALEH and AQEH extracts of roots in Excision model.**



**Fig. 2. Period of Epithelization in excision wound model treated by *E. hookeriana* ointments.**

#### 4.2. Incision model

In the incision repair model the breaking strength of the wounds after topical application of the extracts has been measured. The breaking strength is the ability of healing wound which is measured experimentally by the amount of force required to disrupt it. In the initial stages wound will be having little breaking strength because the clot alone will be holding the edges together. Thereafter breaking strength increases rapidly as collagen deposition increases and cross-linkages are formed between the collagen fibers. The observed tensile strength of the 5% AQEH ointment, 5% ALEH ointment and Standard nitrofurazone ointment were 710.83, 528.33 g and 697.50 g respectively (Fig. 3).



**Fig. 3. Effect of Tensile strength in wound healing activity of *E. hookeriana* by Incision wound model.**

In the study, the results were more encouraging in that the AQEH 5% ointment exhibits better activity than that of standard Nitrofurazone ointment. The order of the activity was found to be AQEH 5% > Standard > ALEH 5%.

When a wound occurs and is exposed to external environment. It is more prone to attack by microbes, which invade through the skin and delay the natural wound-healing process. The significant antibacterial effect of the extracts against the bacterial pathogens *B. subtilis*, *B. cereus*, *S. aerues*, *E. coli*, *P. vulgaris* and fungal sp. Like *A. flavus*, *A. niger*, *C. albicans*. Etc. Confirmed<sup>[20]</sup> that the compounds present in the crude extract are responsible for the effective antimicrobial activity. Further, *Eriolaena hookeriana* possesses potent anti-inflammatory and moderate analgesic properties.<sup>[21]</sup> The external application of the extract on wound prevented the microbes to invade through the wound, resulting protection of wound against the infection of the various organisms. Figure 1 shows the closure of the wound on 16<sup>th</sup> day of treatment with both ALEH and AQEH ointments. *Eriolaena hookeriana* possesses potent anti-inflammatory, moderate analgesic and with anti bacterial properties. Hence, the use of *E. hookeriana* for various skin infections is justified by this work.

#### 5. CONCLUSION

The percentage yield of the extracts was 4.5% w/w, 3.6% w/w for ALEH and AQEH respectively. The preliminary phytochemical screening studies revealed the presence of chemical constituents like flavonoids, saponins, tannins, phenols and alkaloids in the methanolic and aqueous root extracts. Total flavonoid content of AQEH, ALEH (1mg) equivalent to 3.56 µg, 3.51 µg respectively of quercetin was detected. The total phenolics content of ALEH, AQEH (1mg) equivalent to 27.5 µg and 31.54 µg respectively of gallic acid. There is no significant change in colour, pH and odour at 4°C and room temperature on 45<sup>th</sup> day of observation in case of both 5% ALEH and AQEH herbal ointments. A very rapid closure of the wound in the both ALEH and AQEH treated groups observed between 4 and 8 days of post surgery ( $p <$

0.01). The period of epithelisation period was significantly ( $P < 0.001$ ) reduced from 24 days (control) to 17 days. In the study, observed an increased breaking strength of skin and the results were more encouraging exhibiting an increase in the breaking strength AQEH 5% ointment exhibits better activity than that of standard Nitrofurazone ointment. The order of the activity was found to be **AQEH 5% > Standard > ALEH 5%**. Hence by the above results it is shown clearly that plant possess wound healing activity thus it justifies use of *Eriolaena hookeriana* in folklore.

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