

EVALUATION OF WOUND HEALING POTENTIAL IN *CRINIUM DEFIXUM* KER GAWL BULBS

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## ABSTRACT

*Crinum defixum* Ker Gawl is a bulbous herb which has a wide geographical distribution in India. In folklore the bulbs of this plant were used in treating wounds hence the main aim was to evaluate the wound healing potential of *C. defixum*. 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 epithelization was studied while in incision wound model, tensile strength of the wound was measured. 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. The results of present study suggest that extracts of *C. defixum* possess potent wound healing activity justifying its use in folklore.

**Keywords:** *Crinum defixum*, wound healing activity, Excision method, Incision method, Tensitometer.

## INTRODUCTION

Wound healing is a complex multifactorial process that results in the contraction and closure of the wound and restoration of a functional barrier where repair of injured tissues occurs as a sequence of events, which includes inflammation, proliferation and migration of different cell types<sup>1</sup>. Wound is a clinical entity and is as old as mankind, often possesses problem in clinical practice. A lot of research has been envisaged to develop the better healing agents and it has been a challenging task to discover healing agents and keep up pace with problems encountered<sup>2</sup>.

The genus *Crinum* belongs to the *Amaryllidaceae* family and comprises approximately 160 species distributed throughout the tropics and warm temperate regions of the world in Asia, Australia, Africa and America. The *Crinum* species have commercial, economical and medicinal importance. *Crinum defixum* Ker Gawl is the one among *Crinum* genus<sup>3</sup>. It is commonly called Bon-naharu (meaning wild garlic) this plant is having many claims in folklore it is used for treating analgesic, inflammations and wound healing activities. Even today it is used as folk medicine in many countries. Moreover *C. defixum* is reported to contain the active constituents such as caranine, crinamine, crinine, galanthamine, galanthine, haemanthamine and hippestrine. Recently a new alkaloid 5 $\alpha$ -hydroxyhomolycorine has also been reported<sup>4</sup>. Ethanolic extract of dried bulbs of *Crinum defixum* has been reported to have the free radical scavenging<sup>5</sup>, analgesic and anti-inflammatory properties<sup>6</sup>. There is no scientific evidence found for the plant in the treatment of wounds. Hence the present study was focused on evaluation of wound healing potential of the plant.

## MATERIAL AND METHODS

## Collection of plant material

*Crinum defixum* Ker Gawl was collected in Warangal district, A.P., India, botanically identified and authenticated by Dr. Raju S Vastvya, Professor, Department of Botany, Kakatiya University, Warangal Andhra Pradesh, India. A voucher specimen (No VCPVSP-CD02) was deposited in Dept. of Pharmacognosy & Phytochemistry, Vaagdevi College of pharmacy, Hanamkonda, A.P, India for future reference. The bulbs were separated carefully, washed thoroughly and coarsely size reduced.

## Preparation of extracts

For the present study, about 100 g of powdered bulbs were extracted successively with various solvents viz., petroleum ether, chloroform, ethyl acetate and ethanol. The filtrate was concentrated in rotary evaporator and the extracts were calculated for their yield and stored in desiccators. The extracts were designated as PECD for petroleum ether, CLCD for chloroform EACD for ethyl acetate and ALCD ethanol extract respectively. And all extracts were subjected to preliminary phytochemical screening using standard procedures<sup>7</sup>.

## Total Flavonoid content

Aluminum chloride colorimetric method<sup>8</sup> was used for flavonoids determination. One milliliter (1 mL) of sample was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum 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 prepared by preparing quercetin solution (2-10  $\mu\text{g/mL}$ ) in methanol. The concentration of flavonoid was expressed in terms of mg/mL.

## Total phenolic content

The amount of total phenolic content of the extracts was determined by Folin-Ciocalteu reagent<sup>9</sup> 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  $\mu\text{g/mL}$ ) for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent.

## Pharmacological Activity

## Animals used

Male Wistar albino rats (150-180 g) were selected for the experiment. Six rats were taken for each group. The rats were used after acclimatization to the laboratory environment for a 7- day period. They were kept in the departmental animal house at  $26 \pm 2^\circ\text{C}$  and light dark cycles of 10 and 14 h, respectively. Animals were provided with rodent diet 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. IAEC/VCP/2011/10/3/6. In the present investigation, the rats were divided into five groups ( $n = 6$ ): Group I was the control, which receive ointment base, Group-II was treated with reference standard (0.2%, w/w Nitrofurazone ointment), Group-III received ALCD ointment 5% (w/w), Group IV received EACD ointment 5% (w/w)<sup>10</sup>.

## 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>10-12</sup>.

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

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

----- (1)

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.

Period of epithelization was also calculated and compared with that of control group.

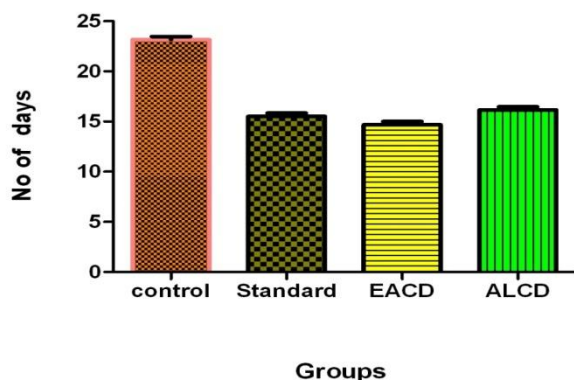


Fig.1: Period of epithelization exhibited by *C. defixum* ointment in excision model

#### Incision wound model

Animals in each group were anaesthetized and one para vertebral-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>13</sup>.

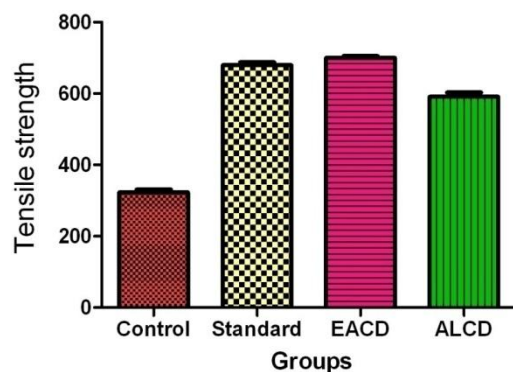


Fig.2: Tensile strength exhibit by *C. defixum* ointment in incision model

#### 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>14-16</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>17</sup>.

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

#### 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 is fixed, were as to another end easy movement of thread was allowed with help of pulley, to the edge of thread weighing balance was attached. Two clamps were tied to the thread in each side. The rats were anesthetized 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.

#### RESULTS

##### Percentage of yield

The percentage yield of the extracts was 0.1, 1.2, 1.8, and 0.9 % for PECD, CLCD, EACD and ALCD respectively.

##### Preliminary Phytochemical Screening

By performing different phytochemical tests it revealed that all the extracts constitutes of alkaloids and tannins. glycosides are present in chloroform, ethyl acetate and ethanolic extracts respectively and were as, ethyl acetate and ethanolic extracts showed presence saponins and tannins, phenols along with flavonoids.

Table 1: Shows preliminary phytochemical screening of *C. defixum*

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

##### Total flavonoid content

Total flavonoid content of EACD, ALCD (1mg) equivalent to 9 µg, 5.09 µg respectively of quercetin was detected.

##### Total phenolic content

In the present study total phenolics content of EACD, ALCD (1mg) equivalent to 47.4µg and 25.08µg respectively of gallic acid was detected.

##### Wound models

Effect of topical application of ointments containing 5% EACD and ALCD extracts. Results obtained from both the wound models have been expressed as Mean ± S.D. and were compared 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 2: Wound contraction area exhibited by *C. defixum* ointment in excision wound model

Post wound day	(% wound contraction)			
	Control	Standard	EACD 5%	ALCD 5%
0	(0)	(0)	(0)	(0)
2	10.1	14.4	10.7	11.1
4	25.7	46.5	52.8	49.4
6	47.4	80	82.4	81.6
8	50.7	88.4	90.3	89.8
10	57.7	94	96.2	95.5
12	61.7	97.7	98.8	97.2
14	70.4	100	100	99

## DISCUSSION

Both ethyl acetate and ethanolic extracts showed the presence of saponins and tannins, phenols along with flavonoids even Total flavonoid and total phenol content also showed rich in phenol and flavonoids which are well known for antioxidants property. Hence we selected these two extracts for the present activity. It is well known that wound healing process is worsen by the free radicals because this reason in most of the cases we observe slow wound healing process hence these extracts containing phenols and flavonoids which exhibits antioxidant are used as wound healing agents.

In excision method both the extracts showed significant wound healing activity but were as Ethyl acetate extract exhibited better wound healing activity when compared to that of ethanolic extract in both parameters measured *i.e.*, wound contraction and period of epithelization. For ethyl acetate extract wound contraction was 100% by 16<sup>th</sup> day as well as period of epithelization was obtained by 14<sup>th</sup> day. The order of wound healing activity was 5% EACD > 2% Nitrofurazone > 5% ALCD. In Incision method tensile strength was found to be 700.2 gm which indicates the presence of good collagen fibers formation.

## CONCLUSION

*Crinum defixum* Ker Gawl is an important medicinal plant having claims for many disorders if we utilize its uses in a systemic and scientific way we can have answer for many disorders. Even in the present study it is concluded that both the extracts of *Crinum defixum* showed significant activity.

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