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WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF ROOT BARK OF *ACHYRENTHES ASPERA* LINN

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Achyranthes aspera, Wound healing, Phytochemical Screening

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ABSTRACT: *Achyranthes aspera* Linn (Amaranthaceae), commonly known as apamarga, is a commonly available plant in India. This plant has been used in treating cuts by people living in various states in India. The Ethanolic extract of root bark of *Achyranthes aspera* was prepared, and its wound healing activity was evaluated. The ethanolic extract of the root bark of *Achyranthes aspera* (10 and 30% w/w applied locally in excised wounds) produced a dose-dependent acceleration in wound contraction and increased betadine content and tensile strength of wounds in rats. The results demonstrate wound healing activity of Ethanolic extract of the root bark of *Achyranthes aspera*.

INTRODUCTION: Wound healing is the process of repair that follows injury to the skin and other soft tissues. It is fundamentally a connective tissue response. The initial stages of wound healing involve an acute inflammatory phase followed by the synthesis of collagen and other extracellular matrix, which are later remodeled to form scar [1]. *Achyranthes aspera* Linn., belonging to the family Amaranthaceae, is commonly found as a weed on wayside and at waste places throughout India. It is known as Apamarg in Sanskrit, Aghedo and Aghedi in Gujarati, Chirchira and Chirchitta in Hindi, and Prickly chaff flower in English. It is widely used for asthmatic cough, snakebite, hydrophobia, urinary calculi, rabies, influenza, piles, bronchitis, diarrhea, renal dropsy, gonorrhea, and abdominal pain [2, 3, 4, 5, 6].

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A powder of dried leaf mixed with honey is useful in the early stages of asthma [9]. One of the drugs from Siddha system of medicine, Naayuruvi kuzhi thailum has *A. aspera* as the primary constituent is reported to be quite effective in the management of asthma [7].

Classification:

Kingdom: Plantae

Subkingdom: Tracheobionta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Caryophyllidae

Order: Caryophyllales

Family: Amaranthaceae

Genus: Achyranthes

Species: Aspera

FIG. 1: *ACHYRANTHES ASPERA* LINN. - CHARCHITAH

TABLE 1: CHEMICAL COMPOSITION OF ROOT BARK OF *ACHYRANTHES ASPERA*

S. no.	Constituents
1	Saponins from alcoholic extract of defatted seeds
2	Oleanic acid from seeds
3	Saponins A and B
4	Saponins C and D from unripe fruits [1]
5	AA, CHO, protein, Fe, Ca, phosphorous
6	Achyranthine, N-methyl pyrrolidine –3 carboxylic acid
7	Water soluble base, betaine
8	Vitamin C
9	Ecdysterone
10	Inokosterone ecdysterone in callus and tissue culture
11	Enzyme level

MATERIALS AND METHODS:

Plant Material: The root bark of *Achyranthes aspera* Linn. (Family: Amaranthaceae) was collected in September 2019 from Jhansi District, (U.P.), India. Dr. Sanjeev Kumar Regional Ayurveda Research Institute (RARI) Jhansi, Uttar Pradesh, India, identified and authenticated the plant. A voucher specimen of the plant was kept in the herbarium of RARI; Jhansi, whose accession number of the specimen is 28696, on 06/08/2020.

Animals: Swiss albino rats (100- 150gm) of either sex housed in standard temperature, humidity, and light (12hr light/ dark cycle). They were fed with a Standard Pellet diet and water *ad-libitum*. The Institutional Animal Ethical Committee (IAEC) of the Institute of Pharmacy, Bundelkhand University, Jhansi U. P. India, approved the experimental protocol. Approval no. BU/Pharm/IAEC/2020/06, per the committee's guidelines for Control and Supervision of experiments on animals, Ministry of Social Justice and Empowerment, Government of India.

Preparation of Extract: The air-dried in-shade plant root bark of *Achyranthes aspera* Linn. was made into coarse powder with grinder. The coarsely powder of root bark was packed in Soxhlet apparatus & continuously extracted with petroleum ether for defatting, which was extracted by ethanol at temp. at 60°-80°C till all the constituents were separated out. The Preliminary Phytochemical screening was carried out on the Petroleum ether and Ethanolic extracts to reveal the presence of phytochemicals in the extracts.

Pharmacological Screening:

Wound Healing Activity:

Excision Wound Model: The animals were divided into five groups of 4 animals each to assess wound healing activity.

Group 1: Received simple ointment base.

Group 2: Received betadine.

Group 3: Received Ethanolic extract of *Achyranthes aspera* roots bark (100mg/kg) suspended in polyethylene glycol.

Group 4: Received Ethanolic extract of *Achyranthes aspera* roots bark (300mg/kg) suspended in polyethylene glycol.

Group 5: Received Ethanolic extract of *Achyranthes aspera* roots bark (500mg/kg) suspended in polyethylene glycol.

Circular wounds of approximately 10 mm in diameter were inflicted on the cleared skin by cutting under the mild Xylocaine 4% topical anesthesia. The areas of the wounds were measured (sq. mm) immediately by using vernier calipers. This was taken as the initial wound area reading.

Group-I animals served as a negative control, which received ointment base I.P. Group-II served as a positive control to which Betadine (5% w/w in

ointment I.P.) was applied topically. Group-III. Group-IV and Group-V animals were similarly treated with the extract EEAA 100 mg, 300 mg, and 500 mg. All the staples were applied once daily. The wound area of each animal was measured on 1st, 4th, 7th, 10th, 13th, and 18th, post wounding day. The wound closure was measured at regular intervals to see the percentage of wound closure and epithelization time that indicate the formation of new epithelial tissue to cover the wound. The number of days required for falling off the scar without any residual of the raw wound gave the period of epithelialization.

TABLE 2: ASSESSMENT OF WOUND HEALING ACTIVITY ON EXCISION MODEL

Groups	4th day	7th day	10th day	13th day	18th day
Groups I (Control)	8.50±0.88	53.36±1.30	81.53±1.48	87.83±1.17	94.48±1.20
Groups II (Standard 5% betadine)	59.53±2.50*	86.27±0.57*	94.85±1.50*	96.80±0.35*	99**
Groups III (test-1)	44.7±4.53**	87.39±1.38*	92.47±1.97**	94.23±0.80**	98.24±0.39**
Groups IV (test-2)	46.7±5.48**	79.62±1.03**	91.79±0.78**	97.70±0.44**	98.70±.089**
Groups V (test-3)	47.8±5.47**	83.89±1.87**	97.50±0.39**	98.56±0.79**	99**

Values were expressed as mean ± SEM for five observations. *P < 0.01, **P < 0.001 versus control; values in parenthesis are percentage closure of original excision wound area.

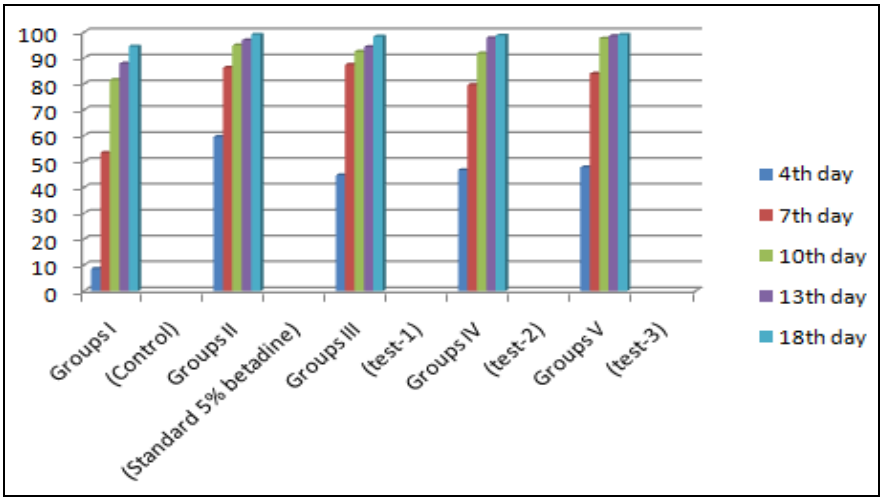

FIG. 2: EFFECT OF EXTRACTS OF *ACHYRANTHES ASPERA* ON EXCISION WOUND MODEL

TABLE 3: EFFECT OF EXTRACTS OF *ACHYRANTHES ASPERA* ON EXCISION WOUND MODEL

Treatment	Excision wound breaking strength (g)
Groups I (Control)	22 ± 0.40
Groups II (Standard 5% betadine)	13.7 ± 0.28*
Groups III (test-1)	18.27 ± 0.47*
Groups IV (test-2)	16.74 ± 0.25*
Groups V (test-3)	15.70 ± 0.25*

Values were expressed as mean ± SEM for five observations. *P < 0.001 versus control.

Statistical Analysis: The results are expressed in mean ± S.E.M. (n=5). Statistical analysis was done by one-way ANOVA, followed by Dunnett multiple comparison test vs. control. P0.05 was considered statistically significant. Wound healing has many events/phases, such as granulation, collagenation, contraction, epithelialization and scar-remodeling [8]. All these phases, except scar remodeling, run concurrently and influence each other. Therefore, it may not be possible to draw firm conclusions about the influence of a given agent on healing by studying only one healing phase [9]. With this in view, the present study was designed to monitor the effects of SRE on different phases of wound healing in rats by employing excisional and incisional wound healing models.

RESULT: The ethanolic extract ointment of *Achyranthus aspera* Linn. Effectively stimulated wound contraction, epithelization, granulation, and collegenation, in comparison with standard drug *i.e.*, betadine ointment (5%w/w) in excision wound model in mice. These results agree with an earlier observation demonstrating wound healing activity of topical application forms roots bark of *Achyranthes aspera* Linn. Increased contraction was also observed in excision wounds in rats by *Achyranthes aspera* extract. Overall, the study

reveals that the ethanolic extract root bark of *Achyranthes aspera* Linn may promote wound healing activity due to its ability to accelerate wound contraction, increased tensile strength, and increased betadine content, suggesting its therapeutic potential in wound healing.

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CONFLICTS OF INTEREST: Nil

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