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Evaluation of Wound Healing Activity of *Echinochloa Frumentacea* Seeds

Anupam Thakur[1], Neelam Painuly [2*], Ashwani Kumar [3]
[1]Research Scholar, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun; anupampharm25@gmail.com
[2]Associate Professor, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun; sopr.neelam@dbuu.ac.in
[3]Assistant Professor, Department of Pharmaceutical Sciences, Gurukul Kangri (Deemed to be University) Haridwar; ashwani@gjv.ac.in
Corresponding Author's: Neelam Painuly; Email: sopr.neelam@dbuu.ac.in

Abstract*; A wound is a loss or breakage of the skin's epithelial cells caused by sub-lethal trauma, leading to disruption of their anatomic and functional continuity. Wound-healing is a process of regaining the integrity of skin cell structures that are affected by microbial or bacterial inflammation and oxidative stress. Echinochloa frumentacea is known for its proven antioxidant, anti-inflammatory, antibacterial, and free radical scavenging properties, but there is no detailed scientific data available regarding its wound-healing activity. The present study was designed to evaluate the wound-healing activity of Echinochloa frumentacea seeds on Wistar albino rats. The wound healing efficacy of the methanolic seed extract of Echinochloa frumentacea was evaluated in the excision wound model at 200mg and 300mg concentrations and compared with the standard drug. The parameters studied include the wound contraction rate and the complete period of epithelialization. The methanolic seed extract of Echinochloa frumentacea was found to possess significant wound healing activity against the control and standard groups, which was evidenced by a decrease in the period of epithelialization and an increase in the rate of wound contraction. As Echinochloa frumentacea seeds have the potential to heal external wounds, it is hypothesized that they may also promote internal wound healing and could serve as a natural alternative in wound management.*
Key-words*: Wound-healing, Echinochloa frumentacea, Methanolic extract, Excision model.*

INTRODUCTION

A wound is a clinical entity and is as old as mankind; effective treatment of wounds is a major challenge for human health. Failure to heal and a prolonged healing time increase the economic and social costs for healthcare professionals, patients, and their families [1,2]. According to WHO statistics, approximately 5 million people could die each year because their wounds do not heal properly.

Skin acts as a protector against external chemicals, dust particles, and diseases. It plays a vital role in wound healing and overall wound management. Wound is a loss or breakage of the skin's epithelial cells caused by sub-lethal trauma leading to disruption of their anatomic and functional continuity [3]. It may occur due to chemical, physical, thermal, microbial, or immunological exploitation of tissues such as accident, surgery, contact with strong acids, fire burn, bacteria, virus, and sometimes caused by the body's immune system [4]. Wounds are classified in various ways by health care professionals. They are divided into external and internal wounds based on the visibility of the injury. External wounds are visible and affect the outer surface of the body, especially the skin. It may represent both acute and chronic wounds based on their healing time. Internal wounds are injuries that occur inside the body (organ/tissue), without visible skin damage. It mainly represents the chronic wounds as having no direct visibility and a long healing time. Acute wounds are mainly traumatic injuries (burns and chemical injuries) and surgical wounds that heal within 4weeks. Chronic wounds that take more than 3months to heal and result in scars [5].

Wound healing or wound repair is the body's natural process that starts with clotting of blood and is completed with the remodeling of cellular skin layers. An imbalance between free radical generation and antioxidants has been detected to induce tissue damage, oxidative stress, and delayed wound healing. Therefore, removing ROS could be an important strategy in healing chronic wounds [6].

The normal wound healing process occurs in four phases, as shown in Fig.1 [7]:

A. Coagulation/Hemostasis, which prevents blood loss

B. Inflammation and debridement of the wound

C. Cellular proliferation

D. Tissue remodeling and collagen deposition.

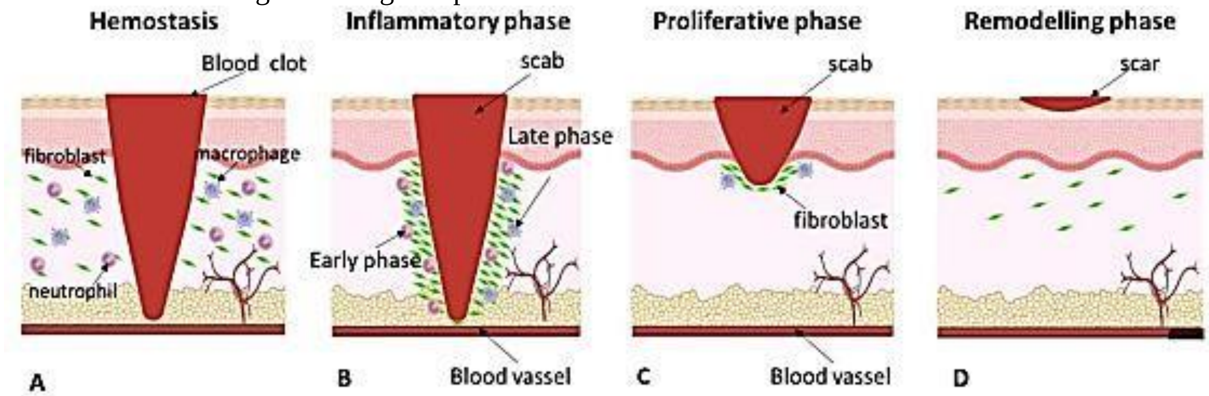

Figure 1: Phases of wound healing.

Various synthetic treatments (analgesics, NSAIDs, and antibiotics) are available in the market for wound management, but sometimes these treatments produce numerous unwanted side effects [8,9]. In recent years, numerous studies have been conducted in the area of wound healing and management through plant-derived medicinal plants [10], and these herbal remedies have demonstrated efficacy in treating wounds as an alternative to currently available synthetic drugs [11]. According to the estimates from the World Health Organization (WHO), 80% of the world's population depends on traditional methods for primary health care, and 85% of this portion uses plants.

Bioactive phytochemicals present in medicinal plants, such as alkaloids, amino acids, fatty acids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds [12], have shown significant potential in the management and prevention of microbial infections and wounds [13]. Phytochemicals responsible for anti-microbial, antioxidant, and wound-healing properties encourage blood clotting, fight with infection, and accelerate wound healing. Medicinal plants rich in phenolic compounds are testified to possess amazing wound healing activity mainly due to their astringent, antimicrobial, and free radical reducing potential [14,15,16]. Flavonoids can also promote excellent wound healing, likely by means of antimicrobial and antioxidant properties, by inhibiting lipid peroxidation that helps in preventing cell damage and enhancing the viability of collagen fibrils [17,18].

A millet *Echinochloa frumentacea* of *Poaceae* family is commonly known as Sawa rice (Hindi) and mainly found in the Uttarakhand Himalayas of India. Its seeds are small, spherical (3-4mm), and brown. It is an excellent choice for diabetics, expectant mothers, and infants as a healthy diet [19]. Recent investigations demonstrated the occurrence of important bioactive phytoconstituents in *Echinochloa frumentacea* seeds like flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, saponins, amino acids, vitamin C, and E [20,21]. The seeds were also found to possess antioxidant [22], anti-diabetic [23], anti-inflammatory [24], anti-bacterial [25], and free radical scavenging activities [26] that play a more helpful and important role as an ideal wound healing remedy. However, the medicinal values of millet for wounds have not yet been reported. The *Echinochloa frumentacea* seeds were selected with the promise that if they reduce the free radicals through their antioxidant and anti-inflammatory activities, then they could have an effect on wound healing also.

The present work focuses on the evaluation of the wound healing activity of the methanolic extract of *Echinochloa frumentacea* seeds topically in excision wound model in Wistar albino rats at 200mg and 300mg concentration and compares it with the standard drug.

MATERIAL AND METHODS

Materials: Fresh seeds of *Echinochloa frumentacea* millet were procured from Garhi Cantt. (Uttarakhand) and was authenticated at Department of Botany, Shaheed Durga Mall Govt. Post Graduate College, Doiwala, Dehradun (Authentication No. SDMPGC/BOT/ID/2024-25/02 Dated 26th May 2025) by comparing morphological evaluation. The herbarium of the millet specimen was deposited at the same.

Preparation of seed extract: The shade-dried seeds of *Echinochloa frumentacea* were coarsely powdered. After being loaded into a Soxhlet extractor, the powder was successively extracted using methanol for 8hrs. The methanolic solvent was removed by using a Hot water bath. The yield (%) of extraction was calculated by Equation 1:

$$\text{Yield of extraction (\%)} = \frac{Weight\ of\ crude\ extract}{Weight\ of\ powdered\ seeds}\ x\ 100$$

Preliminary Phytochemical Screening: The methanolic seed extract of *Echinochloa frumentacea* was tested qualitatively for different phytochemicals using various phytochemical screening tests, using a standard procedure as shown in Table 1 [27]:

Table 1: Qualitative phytochemical screening test

S. NO	Phytochemicals	Phytochemical Screening Tests
1.	Alkaloids	Wagner's Test: Mix gently a few drops of Wagner's reagent and 2-3mg of extract. Reddish-brown or brown precipitate confirms the occurrence of alkaloids.
2.	Amino Acids	Ninhydrin Test: Heat the mixture of 2ml of ninhydrin solution with 1-2mg of extract. A deep blue or purple color indicates the existence of amino acids.
3.	Cardiac Glycoside	Bromine Water Test: Mix a small amount of bromine water & extract. Yellow precipitate indicates the presence of cardiac glycosides.
4.	Fatty Acids	Litmus Test: Spot a few drops of extract on blue litmus paper. Turning blue litmus into red shows the presence of fatty acids.
5.	Flavonoids	Lead Acetate Test: Gently shake the mixture of 2ml extract and 1-2ml of 10% lead acetate solution. Formation of a yellow or cream-colored precipitate shows the existence of flavonoids.
6.	Phenolic Compounds & Tannins	Lead Acetate Test: Gently shake the mixture of 2ml extract and 1-2ml of 10% lead acetate solution. Formation of a white or yellowish precipitate indicates the occurrence of phenolic compounds and tannins.
7.	Terpenoids	Salkowski Test: Add 2ml of chloroform to 2mg of extract in a test tube, then carefully add 3ml of concentrated sulfuric acid down the test tube's side to form a layer. Terpenoids are shown by a reddish-brown coloring at the interface of two layers.
8.	Saponins	Froth Test: Shake the test tube after adding 5-10ml of distilled water to the extract (after evaporating the methanol). Formation of froth or foam that lasts for at least 10mins indicates the presence of saponins.

Animals: The healthy Wistar albino rats (100-150g) of either sex, housed under standard environmental conditions of temperature and humidity (25±0.50◦C) and 12h light/dark cycle were utilized for the studies. The animals were fed with a standard pellet diet and water ad libitum. The animal studies were performed at Animal House, Gurukul Kangari Vishwavidyalaya, Haridwar, with due permission from the Institutional Animal Ethical Committee (registration no. 704/CPCSEA, India dated 25/8/2003).

Acute dermal toxicity: The study was performed to found out the therapeutic dose of the methanolic extract. The acute dermal toxicity testing of the methanolic extract was done by directly applying the highest concentration of 300mg on the shaved back of the rats. The OECD guidelines no. 402 (OECD guidelines, 1987) were followed for the study.

Wound healing activity: The animals were divided into three groups with six animals in each group, i.e., control, standard, and test. The control group was untreated with any ointment or extract; the Standard group was treated with (CiplaHealth containing 5% (w/w) povidone-iodine, Batch no. N0241043) Betadine ointment 5%(w/w); and the Test group received extract-based treatment at different concentrations as mentioned below:

Test group A: 3 animals received 300mg of extract-based treatment.

Test group B: 3 animals received 200mg of extract-based treatment.

Excision wound model: The anesthesia ketamine (0.5ml/kg via the i.p route) will be administered before shaving the dorsal thoracic region of healthy rats. The excision wound of a circular area (approx. 400mm^2) & 2mm depth will be given on the ethanol-sterilized dorsal thoracic region of rats as described by Morton and Malone (1972) [28]. On wounding day (day 0 shown in Fig.2), wounds are left exposed to the open environment. The topical preparations are applied to wounds every day until they are completely healed. On 04, 08, 12, and 16 post-wounding days, the area of the wound was measured, and the mean wound closure % is reported in Table 3.

Figure 2: Circular excision wounding day 0.

The period of epithelialization was calculated as the number of days required for the wound to become fully covered with epithelial cells [29]. Wound healing rate was calculated by Equation 2 [30]:

$$\%\ of\ wound\ closure = \frac{\text{wound area on day 0} - \text{wound area on day n}}{\text{wound area on day 0}}\ x\ 100$$

RESULT

Yield of Extraction: 80g of seed powder was loaded into a Soxhlet extractor with 400ml of methanol. After removing the methanol via hot water bath, 2.14g of crude extract was obtained. By calculating through equation (1), the yield (%) of extraction was found to be 2.67%.

Phytochemical Analysis: The preliminary phytochemical investigation of the methanolic seed extract of *Echinochloa frumentacea* revealed the presence of alkaloids, amino acids, fatty acids, flavonoids, phenolic compounds, tannins, terpenoids and saponins. The amino acids, fatty acids, flavonoids, and phenolic compounds are present in higher quantities as they indicate a proper result. The cardiac glycosides are not present in it.

Table 2: Result of qualitative phytochemical analysis.

S. NO	Phytochemical Test	Result
1.	Alkaloids	(+)
2.	Amino Acids	(+)

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3.	Cardiac Glycoside	(-)
4.	Fatty Acids	(+)
5.	Flavonoids	(+)
6.	Phenolic Compounds & Tannins	(+)
7.	Terpenoids	(+)
8.	Saponins	(+)

Acute Dermal Toxicity Studies: In the acute dermal toxicity studies, no signs of toxicity or mortality were observed. Therefore, the 300mg and 200mg doses were taken as the therapeutic doses.

Excision wound study: The result of excision wound model are presented in Table 3 Fig.3. The values presented in the table represents percentage wound healing at 04, 08, 12, 16 days for control (untreated), standard (betadine treatment group) and the test group (200mg and 300mg (w/w)). It is observed that the wound contracting ability of animals treated with 300mg (w/w) extract was found to be higher on days 08, 12, and 16 as compared to the control and standard group. The animal treated with 300mg (w/w) methanolic seed extract had the shortest epithelization period, that is 17 days.

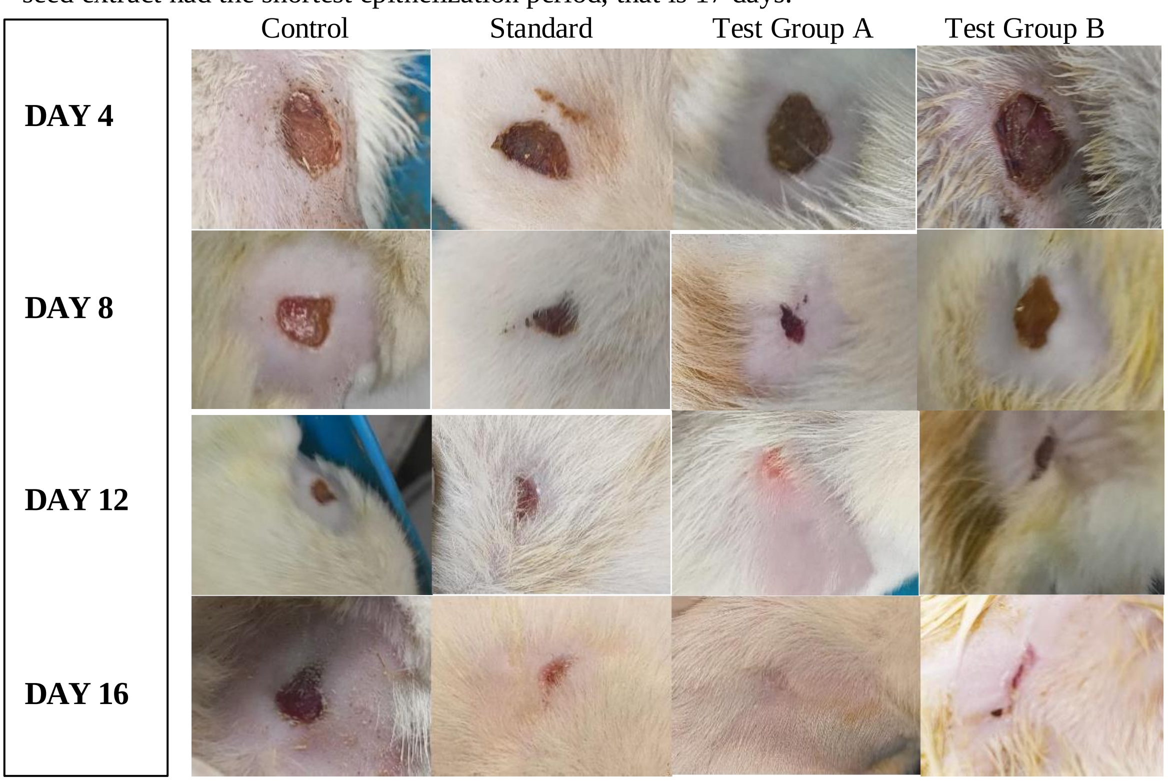

Fig. 3. Different stages of excision wound healing measured at different time intervals.

Table 3: Topical effect of methanolic seed extract of *Echinochloa frumentacea* on wound contraction of excision model.

GROUP	DAY 4th	DAY 8th	DAY 12th	DAY 16th	PEROID OF EPITHELIZATION (DAYS)
Mean (%) ± SEM					
CONTROL	13.61±3.72	52.77±5.15	70.93±3.9	80.76±2.02	24
STANDARD	11.35±3.51	65.83±4.74	80.83±1.86	85.01±1.26	21
TEST group A	16.79±2.27	74.99±3.13	94.57±0.5	100***	17
TEST group B	11.29±3.56	61.43±3.15	84.05±1.04	94.78±0.49	19

n = Each group has 6 animals: test groups A and B have 3 animals each.

Student t-test are used to compare test groups with the control and standard groups.
*** P < 0.001

CONCLUSION

The principle of wound healing to regain the anatomical continuity and function of the affected skin is to lessen tissue damage and provide suitable tissue perfusion and oxygenation, proper nutrition, and a moist wound healing environment. The result of the excision wound model indicates that in the first 4days, no increase in wound contraction has been seen in any groups. The results of the 8th and 12th day suggest that there is a considerable increase in the wound contraction in the group treated with 300mg (w/w) methanolic extract, revealing that the extract can induce cellular proliferation. The wound healing studies on seeds of *Echinochloa frumentacea* indicate that the phytochemicals, which are responsible for its antioxidant, anti-inflammatory, antimicrobial, and free radical scavenging activities, play a prior role in the external wound healing process, and its topical application could serve as a natural alternative in wound management. It treats the external wound healing as proven above; their oral consumption through food supplements could be effective in internal tissue damage and build a natural replacement therapy against synthetic dosage formulation. Further studies are needed to validate their effectiveness in internal wounds or tissue damage, which will boost the economy of Uttarakhand.

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