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RESEARCH ARTICLE

Development and Evaluation of *Amaranthus spinous* Methanolic Extract Ointment for *In Vivo* Wound Healing Properties in Wistar Rats

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Abstract

Background: Traditional herbal medicines are minimally processed, plant-derived substances used in local treatments, such as tinctures, teas, poultices, and powders. The herb *Amaranthus spinosus* that is full of polyphenols, crude fiber, proteins, and saccharides is very effective for many diseases. **Aim:** Traditional medicinal herb of *A. spinosus* having wound healing potential with well-known medicinal value. The study involves the formulation of an ointment prepared with the methanolic extract of this plant and its phytochemical analysis for the identification of bioactive compounds. **Methods:** Its efficacy will be evaluated by the *in vivo* studies on controlled wounds in animal models and comparison of results with standard treatments. It will also assess the safety of the ointment for topical use and the general effectiveness of the ointment in wound healing. The rationale, therefore, remains that if it is able to establish a scientific validation for the traditional use of *A. spinosus* in the management or healing of wounds, then one more avenue of an effective treatment option will be opened. **Result:** By using ANOVA method wound shrinkage values for the control group ranged from 10.82±0.68 to 68.69±2.60 by the 16th day. In the case of Test-I, where the concentration used was 5%, the increase has been from an initial value of 11.24±1.23 to 76.12±1.93 on the 16th day. Test-II, where the concentration used was 10%, the increase has been from 14.14±0.98 initially to 93.99±0.68 on the 16th day. Treatment of the standard group with Soframycin was initiated at 15.26±1.25 and was completely closed with the values of 100±0.75 on day 16. **Conclusion:** *In vivo* model studies revealed that the methanolic extract of *A. spinosus* showed a very strong acceleration of wound healing without causing irritation or toxicity on the skin.

Keywords: *Amaranthus spinosus*, Excision Model, Methanolic Extract, Ointment, Wound Healing

1. Introduction

It is the organ that covers the human body from head to toe and protects it from pathogenic invasion. It prevents excessive loss of fluid from the body, protects the body, and acts as a thermal barrier[1]. Thus, in the event of a wound, this multi-functional organ needs to get back into its functioning. Maintenance of skin integrity or repairing of damaged tissues is a multi-compiler biochemical process involving hemostasis, proliferation, inflammation, and remodeling[2]. Hemostasis, inflammation, proliferation, and remodeling are the four phases of the intricate biochemical process that constitutes wound healing. The local factors that affect the process of wound healing are diminished oxygenation, infections, and antibiotics. Age, gender, sex hormones, stress, and ischemia are examples of systemic variables and more[3]. The extracellular matrix helps angiogenic capillaries, which provide the vital nutrients and oxygen that injured cells require to repair, therefore speeding up the healing process[4]. Wounds that have stalled in some part of normal healing stages are the ones referred to as chronic wounds, resulting in pathologic inflammation. Thus, delayed, incomplete healing that is not coordinated and often

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associated with poor anatomic and functional results ensues[5]. In conclusion, oxygenation is an essential local component that inhibits wound infection, encourages angiogenesis, boosts collagen synthesis, and facilitates wound contraction[6]. However, as the cell is supplied with the necessary energy, oxygen is consumed by oxidative phosphorylation, forming reactive oxygen species. Overproduction of ROS has the potential to cause oxidative stress at the wound site and complicate the healing process[7,8]. The plant in herbal medicinal plants possesses biological compounds that act in the facilitation of healing and are able to treat wounds, skin diseases, cuts, and even burns. Plant metabolites, having the properties of tissue regeneration and healing, are rich in polyphenols with high antioxidant activity and phytochemicals appropriate in addressing different types of skin damage[9]. Medicinal herbs slave stands valuable fountains of wound therapy due to their many biological activities that include antioxidant, antimicrobial, anti-inflammatory, and anticancer activity, among others[10]. Phytomedicine involves using components of medicinal plants for treatment. Historically practiced outside conventional medicine, it has recently gained main stream acceptance due to advancements in analysis, quality control, and clinical research. Herbal medicine is defined by the World Health Organization (WHO) as the application of traditional knowledge and practices from ancient times to the treatment of illnesses via the use of plants. Traditional herbal medicines are minimally processed, plant-derived substances used in local treatments, such as tinctures, teas, poultices, and powders. The use of plant medicine dates back to prehistoric times and has contributed to the foundation of modern medicine[11]. Consequently, natural plants, with healing features, could be considered a better option than existing pharmaceuticals on the basis of the scientific evidence to support them. The herb *A. spinosus* that is full of polyphenols, crude fiber, proteins, and saccharides is very effective for many diseases. Formerly, it has been employed to mend sores, get the inflammation down, mitigate manipulation, cure the depression, and even save the lives of those who suffer from malaria[12]. *A. spinosus*, also called Kate Wali Chaulai in Hindi, is a tropical vegetable that is grown in India, Sri Lanka, and other countries (Figure 1)[13].

2. Materials and Methods

2.1 Collection and Authentication

Plant was collected from locality of NIMS University in the month of December. This plant is authentic by botanical survey of India, Kolkata, West Bengal (Ref no- BSI/DRC/2022-23/Tech/436).

3. Pharmacognostical Studies

3.1 Microscopic Studies of Leaf

Through the utilization of sense organs for plant material evaluation, the samples that had been required from the location where the plants were found were collected and fixed with 90% v/v ethanol (FAA), 5 ml of formalin, and 5 ml of acetic acid. The specimens had been dehydrated using tertiary-butyl alcohol in a graded sequence following a 24-hour fixing period[14]. To cut to the chase, rotary microtomes had already been employed for chopping paraffin-embedded specimens into 10-12 micrometer sections. The sections had undergone further processing after typical dewaxing techniques[15,16].

3.2 Physical Studies

Total ash, water-soluble ash, acid-insoluble ash, alcohol-soluble extractive, water-soluble extractive, and moisture content were the main physicochemical parameters of *A. spinosus* powder that had been



Figure 1. *Amaranthus spinosus* plant.

evaluated. According to the study, these crucial traits had been noted and observed (Table 1)[17].

3.3 Extraction Process

The *A. spinosus* had been extracted using a Soxhlet apparatus with methanol as the solvent. This extract had been drained into the china dish, and concentration had begun in a hot water bath. Yield was calculated when the crude product was obtained. This was followed by the qualitative and phytochemical investigation of the methanolic extract of *A. spinosus*[18].

4. Qualitative Preliminary Phytochemical Detection

The phytochemical screening was carried out to detect the various phytochemical constituents by standard procedures[19-21].

5. Development of Ointment Formulation

The 10% ointment contained 2 grams of the methanolic extract of *A. spinosus* while the 5% ointment contained 1 gram. The emulsifying wax quantity was 28 grams in case of 10% and 14 grams in case of 5%. The amount included of paraffin liquid was in composition. The taken amount is 70 ml in case of 10% ointment and 35 ml in case of 5% ointment[22].

5.1 Ointment Assessment Parameters

5.1.1 Color

Every ointment had been evaluated visually to determine its color and fragrance[23].

5.1.2 pH

The pH of ointment formulation was determined by using a digital pH meter. The two-hour passed after adding one gram of ointment into 100 milliliters distilled water solution. The average pH value was noted after performing three measurements[24].

5.1.3 Spreadibility

It had been defined as the time taken in seconds for two slides to have separated from each other after the cream had been placed between them and a given load had been applied. The higher the spreadibility was obtained by the better separation time.

It is computed using the formula $S = M \times L/T$, where M is the weight fastened to the top slide, S is the formulation's spreadibility, L is the glass slide's length, and T is the time required to separate the slides[24].

5.1.4 Extrudability

A collapsed ointment tube was sealed by a crimped end, very tightly squeezed. The extrusion of the ointment had been allowed to continue until pressure had been released, after the removal of the cap. Then it had been measured how much weight, in grams, it took to extrude an ointment ribbon of 0.5 cm in 10 seconds[25].

6. Wound Healing Animal Biological Screening

6.1 Experimental Animals

The protocol of the experiment was approved by Institutional Animal Ethics Committee (IAEC-NIMSUR/IAEC-02/2022/06) at NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan and was conducted for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

6.2 Excision Wound Model

Wound contraction and epithelization had been investigated in excision wounds. The animals had been anaesthetized with diethyl ether; their hair had been removed from the back with sterilized razor blades, a circular incision measuring approximately 300 square millimeters in area and 2 millimeters deep had been made 5 centimeters from the ear in the depilated dorsal thoracic region; and the laceration had remained exposed. Wound tracings were taken on the day of inciting the wound and then on alternate days till the complete healing cycle of the wound was achieved. They had used a permanent marker on translucent tracing paper. Measurements of the wound areas had been taken for every group on days 1, 4, 8, 16, and 20[26,27].

6.3 Animal Treatment Protocol

There had been a total of 24 rats when the rats with excision wound models had been randomly assigned to four groups, each consisting of six rats. The following

treatments had been given. There had been four groups of six animals each. Group I had been the control group and had been given normal saline. Group II received the ointment of 5% *A. spinosus*, whereas Group III had an ointment of 10%. The standard drug used was Soframycin for Group IV[28].

7. Result

7.1 Gross Anatomical Studies of Leaf

The vascular bundle had a somewhat asymmetrical shape and was of a moderate size. It was collateral, with phloem on the abaxial side and xylem on the adaxial side. One or two protoxylem elements were grouped with large, thick-walled, angular metaxylem elements in the xylem.

7.2 Qualitative Preliminary Phytochemical Screening of *A. spinosus*

The phytochemical screening of methanol and aqueous extract of *A. spinosus* is shown in Table 2.

7.3 Physiochemical Evaluation Parameter for Ointments

The ointment assessment parameters are shown in Table 3A and 3B.

7.4 Statistical Analysis

One-way **ANOVA** was followed by Bonferroni's multiple comparison test with equal sample size. The difference was considered significant when $p<0.01$. All values were expressed as mean ± S.E.M.

7.5 Wound Healing Activity

Measurements of the wound areas had been taken for every group on days 1, 4, 8, 16, and 20 are expressed in Table 4 and Figure 2.

Table 1. Physiochemical parameter of *A. spinosus*

S. No.	Parameters	Value Percentage of weight/weight
1	Moisture content	9.07
2	Total ash value	11.60
3	Acid insoluble ash value	1.90
4	Extractive value of water	11.03
5	Methanolic extractive value	6.60
6	Determination of foreign matter	Nil

8. Discussion

In the present study, the effectiveness of *A. spinosus* on wound healing has been explored. This process was developed in view of the historical importance of the plant, *A. spinosus*, in India. Medicinal plants like *A. spinosus* is an important role in treating different conditions in animals[29]. Flavonoids, secondary

Table 2. Phytoconstituents screening evaluation of different extracts of *A. spinosus*

S. No.	Chemical Test	Methanolic Extract	Aqueous Extract
1	Wagner's test	-	-
2	Dragendorff's test	+	+
3	Shinoda test	-	+
4	NaOH test	+	+
5	Keller-Kailani test	-	-
6	Phenol tests (Ferric chloride test)	+	+
7	Foam test	-	-
8	Gelatin test	-	-
9	Molisch's test	+	-
10	Fehling test	-	-
11	Millon's test	-	-

+ denotes presence, - denotes negative.

Table 3A. *Amaranthus spinosus* methanolic extract ointment development process evaluation

S. No.	Parameters	Observations
1	Color	Whitish in color
2	Odor	Petroleum like jelly
3	Consistency	Smooth
4	Spread ability	7
5	Solubility	Soluble in boiling water
6	Extrudability	12.5
7	Washability	Moderate
8	Non-irritancy	Non-irritant

Table 3B. *Amaranthus spinosus* ointment average value of pH

S. No.	Parameter	Observation	Average Value
1	Potential of hydrogen (pH)	6.09	6.13
		6.17	
		6.14	

Table 4. Effect of *A. spinosus* ointment on healing by excision wound model

	Wound Shrinkage					
Class	**Initial**	**4th**	**8th**	**12th**	**16th**	**Complete Recovery (Days)**
I (Control)	10.82±0.68	18.82±0.68	38.12±1.80	48.21±1.80	68.69±2.60	24
II (Test-I) 5%	11.24±1.23	24.22±1.42	48.94±1.24	66.92±0.13	76.12±1.93	21
III (Test-II) 10%	14.14±0.98	35.14±0.54	52.14±0.85	75.12±0.79	93.99±0.68	19
IV (Standard) Soframycin	15.26±1.25	42.24±1.07	65.34±1.70	90.12±1.08	100±0.75	16

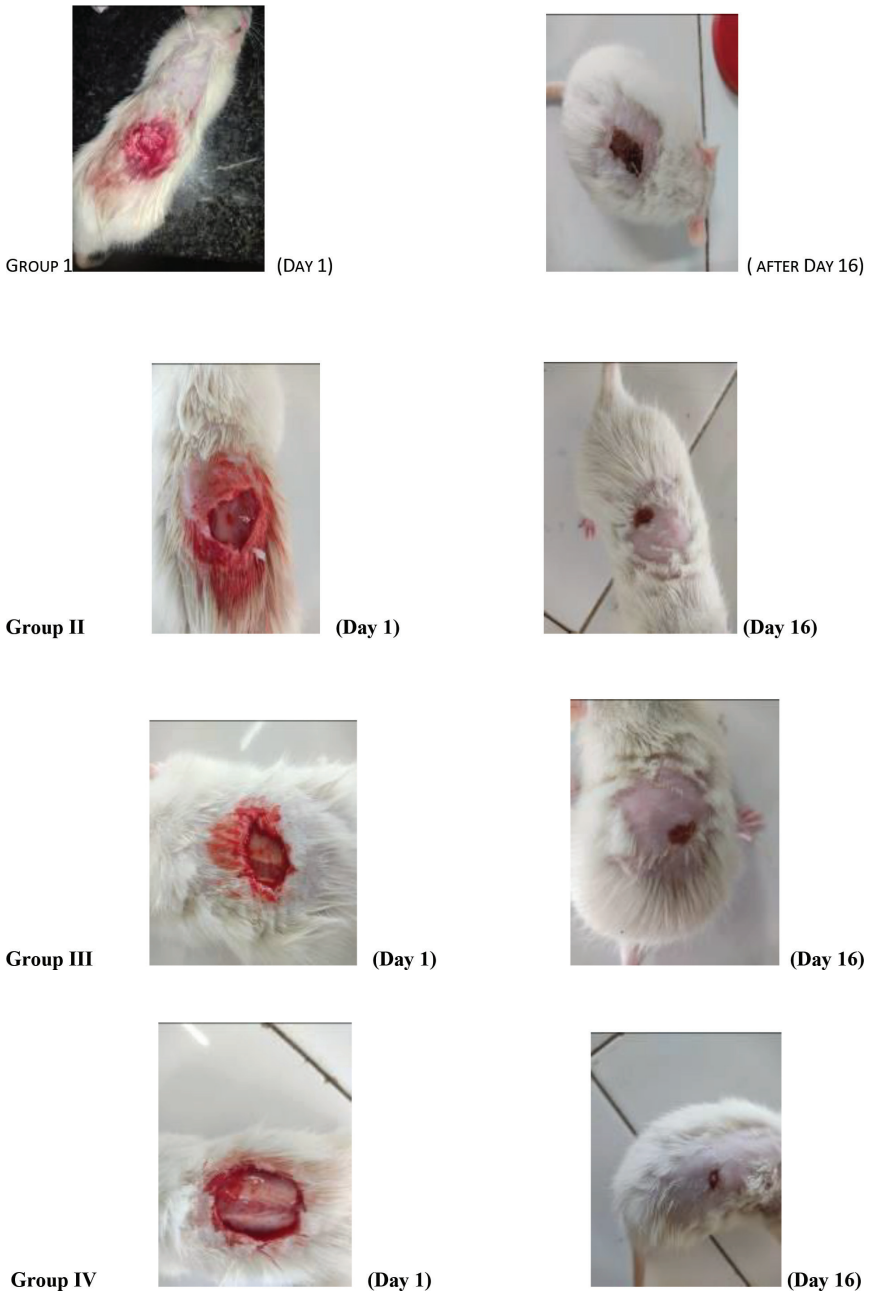


Figure 2. Result after 16 days of *A. spinosus* ointment applied on four groups like Group I was the control group - normal saline. Group II - 5% ointment of *A. spinosus*, while Group III - 10% ointment. The standard drug used was Soframycin for Group IV.

metabolites with a 15-carbon structure, are vital for wound healing due to their antioxidant, anti-inflammatory, and antimicrobial effects. They accelerate healing by neutralizing oxidative stress, promoting new blood vessel formation, and modulating inflammation, making them important in both traditional and modern medicine for enhancing tissue repair[30]. The physicochemical standardization of *A. spinosus* revealed that the total ash content 11.60% and acid insoluble ash 1.90%, this indicates a significant presence of soluble minerals, beneficial for nutritional and therapeutic applications. Additionally, the moisture content was measured at 9.07%, suggesting a relatively low water level, which can enhance shelf life and reduce microbial contamination. Overall, these findings highlight the quality and potential uses of *A. spinosus* in herbal medicine and natural products[31].

A clean pathway represents the smooth conduct of extraction process by extractive value show as water and methanolic respectively 11.03 and 6.06%, i.e effectively shows that methanolic extraction is basis pathway for *A. spinosus*[32]. Phytochemical screening revealed that the extracts contain carbohydrates, flavonoids, and alkaloids, each contributing to the plant's health benefits. Carbohydrates provide energy and support body functions, while flavonoids and alkaloids offer antioxidant, antimicrobial properties[19].

Ointment development assessment the various parameter like pH, extrudability, spread ability respectively 6.13, 12.5, and 7.0. The wound shrinkage in four different classes was measured for 16 days. The values of wound shrinkage in the control group Class I were 10.82±0.68, initially; on the 4th day, it was 18.82±0.68; on the 8th day, 38.12±1.80; on the 12th day, 48.21±1.80, and on the 16th day, 68.69±2.60. The wound shrinkage values for the Test-I group, Class II, treated with the 5% concentration, were 11.24±1.23 initially; on the 4th day, it was 24.22±1.42; on the 8th day, 48.94±1.24; on the 12th day, 66.92±0.13; and on the 16th day, 76.12±1.93. The wound shrinkage values for Test-II group of Class III at a concentration of 10% were 14.14±0.98 at initiation, 35.14±0.54 on the 4th day, 52.14±0.85 on the 8th day, 75.12±0.79 on the 12th day, and 93.99±0.68 on the 16th day. The wound shrinkage values in the standard group, treated with Soframycin, finally reached 15.26±1.25 initially, 42.24±1.07on the 4th day, 65.34±1.70 on the 8th day, 90.12±1.08 on the 12th day, and 100±0.75 on the 16th day.

9. Conclusion

Studies on *in-vivo* models demonstrated the presence of rapid wound closure with the plant methanolic extract of *A. spinosus*, no skin irritation or toxicity was noted with the plant extract. When applied on the animal skin at a concentration of either 5% or 10%, the application of *A. spinosus* greatly accelerated the process of wound healing. The ointment showed quite impressive wound healing property of *A. spinosus*. Thus, extract of *A. spinosus* may serve as an effective alternate herbal therapy for wound treatment.

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