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Evaluation of Wound Healing Activity of Aqueous Extract of *Chromolaena odorata* on Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: A wound can be defined as any physical injury or break in the skin or underlying tissues caused by trauma, surgery, or various medical conditions. Wound healing is a complex and dynamic biological process that occurs in response to injury or damage to the skin or underlying tissues. Medecinal plant have been reported to show wound healing Potential via angiogenesis activation of NF-κB, favoring pro-inflammatory cytokines, increased expression of induciblenitric oxide syntheses (iNOS) and alpha 1 type 1 collagen, and anti-oxidant activity.
Materials and Methods: The Present Research work Conclude the wound healing potential of standard and aqueous extract of *Chromolaena odorata* was studied on Wistar rat excision wound model.

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Results and Discussion: The topical application of standard (soframycin) showed a significantly faster wound contraction rate (70.58%) on 15th post-operative day as compared to non-treated group (33.33%).
Conclusion: The topical application of standard (soframycin) showed a significantly faster wound contraction rate (70.58%) on 15th post-operative day as compared to non-treated group (33.33%). Aqueous extract of *Chromolaena odorata* showed wound contraction rate of (61.66%) on 15th post-operative day as compared to non-treated group (33.33%).

Keywords: Wound; angiogenesis; cytokines; excision; heal.

1. INTRODUCTION

A wound can be defined as any physical injury or break in the skin or underlying tissues caused by trauma, surgery, or various medical conditions.Wound healing is a complex and dynamic biological process that occurs in response to injury or damage to the skin or underlying tissues. It is a remarkable sequence of events involving various cellular and molecular mechanisms aimed at repairing and restoring the integrity of the injured tissue. The process of wound healing can be broadly categorized into three overlapping phases: inflammation, proliferation, and remodeling. During the initial inflammatory phase, damaged blood vessels constrict to prevent excessive bleeding, and immune cells migrate to the wound site to clear debris and defend against potential infections. Inflammatory mediators and growth factors are released, triggering the recruitment of specialized cells that contribute to the subsequent phases. Next, the proliferative phase involves the formation of new blood vessels (angiogenesis) to supply oxygen and nutrients to the healing area. Fibroblasts, a type of cell, produce collagen and other connective tissues to rebuild the damaged area. Epithelial cells at the wound edges multiply and migrate to cover the wound, forming a new outer layer of skin. Finally, the remodeling phase occurs as the wound undergoes structural and functional changes. The newly formed collagen fibers are reorganized and strengthened, and the scar tissue gradually matures. This phase can take months or even years, with the scar becoming more refined and less noticeable over time. Several factors can influence the wound healing process, including age, overall health, nutritional status, and the presence of chronic diseases like diabetes [1,2].

"Chromolaena is being used traditionally for its many medicinal properties, especially for external uses as in skin infections, inflammation etc. Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties. The plant contains active constituents like phenolic acids (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic and vanillic acids) and complex mixtures of lipophilic flavonoid aglycones (flavanones, flavonols, flavones and chalcones) are major and powerful antioxidants showing pharmacological activity like wound healing" [3].

2. MATERIALS

2.1 Drugs and Chemicals

"The leaves of *Chromolaena odorata* are collected locally from wild source of (Berhampur, Odisha), Ethanol, processed plant materials, soframycin (Sanofi Aventis Pharma), Bupivacaine (Marcaine), sodium lauryl sulphate, propylene glycol, stearyl alcohol, white petrolatum, Dragendorff's reagent, tartaric acid, potassium iodide, alpha-naphthol, acetic anhydride, hydrochloric acid, sulfuric acid, ferric chloride, soxhlet apparatus" [4,5].

2.2 Animals

Male Wistar strain rats were used, weighing between 100-120 g. The animals were acclimatized for 7 days under standard animal husbandry condition. i.e., Room temperature - 19.2˚C to 23.9˚C, Relative humidity - 45-55%, Light/Dark cycle - 12:12 hr, *ad libitum.*

3. METHODS

3.1 Extract Preparation

Leaves of *Chromolaena odorata* were air dried in the shade followed by converting it to coarse powder form. The extracts were prepared using 100gm prepared coarse powder of leaves by successive solvent extraction method, using purified water (ratio 1:8 of solvent, soxhlation time 72 hrs). The extracts obtained were concentrated using rota-evaporator. The concentrated extracts were then evaporated to dryness, in vacuum oven at temperature not

more than 50˚c. The dried extracts were then stored at 2-8˚C in refrigerator.

3.2 Preliminary Screening of Phytochemicals

The prepared extract was further subjected for the preliminary screening of phytochemicals which included tests for the presence of alkaloids, carbohydrate, flavonoid, saponin, steroids and terpenoids, tannins and phenolic compounds.

3.3 Preparation of Ointment Base

The ointment base was prepared using sodium lauryl sulphate (0.1g), propylene glycol (1.2g), stearyl alcohol (2.5g), white petrolatum (2.5g), purified water (3.7ml). The stearyl alcohol and white petrolatum was melted on a hot plate and the mixture was heated upto 70˚C.

The remaining ingredients were dissolved in water with the aqueous extract and the solution was heated to 70˚C. Further the oleaginous phase was slowly added to the aqueous phase, stirring constantly. Finally the mixture was removed from the heat and was stirred until it congeals [6,7].

3.4 Experimental

Creation of wound: Excision wound model was used for wound healing evaluation. Skin of the dorso lateral flank area of the Wistar rats were depilated with a depilatory cream one day prior to wound creation and disinfected with 70% v/v alcohol. Rats were anesthetized with Bupivacaine (Marcaine) (0.5%) s.c injection. The skin from the predetermined shaved area was excised to its full thickness to obtain a wound area of about 2 cm. Excision wound was inflicted on the dorsal region 1-1.5 cm away from vertebral column. Homoeostasis was achieved by blotting the wound with cotton swab soaked in normal saline.

The animals were then randomly divided into three groups of six each. Group I being non-treated control group. Group II was the standard group and was treated with Soframycin. Group III was the test group, treated with successive aqueous extract ointment.

Standard wound cleansing was performed daily for all the groups with normal saline prior to application of drugs. Treatments were given once daily, till complete healing. For measuring the progressive changes in rate of the wound contraction, the wounded area were photographed at every 3 days interval from day 0. The change in excision wound area were measured in cm. The changes in the wound size were expressed as percentage contraction of the original wound size at 0^{th} day and calculated by the formula given below:

$$\text{Wound contraction (\%)} = (A_0 - A_n \div A_0) \times 100$$

Where: A_0 is wound area on 0^{th} day, A_n is wound area on n^{th} day

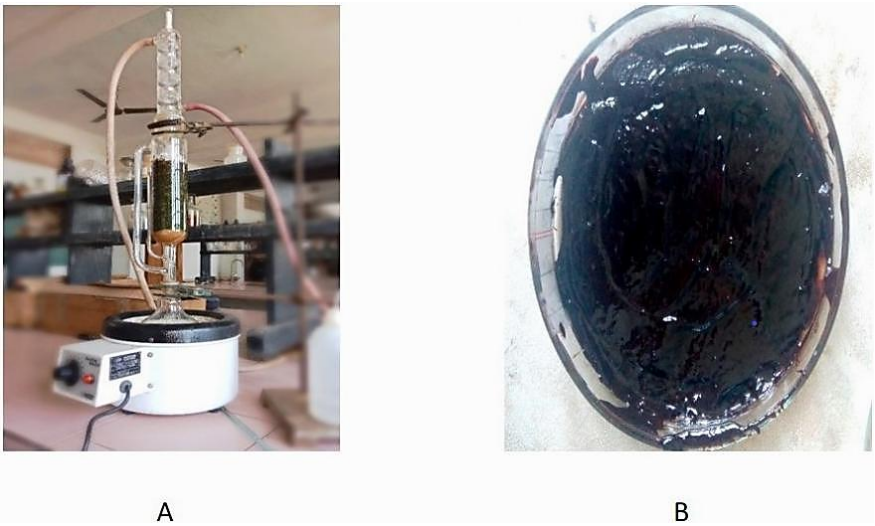

Fig. 1. (A) Soxhlet extraction of leaves of *Chromolaena odorata* (B) Successive aqueous extract of *Chromolaena odorata* [8,9]

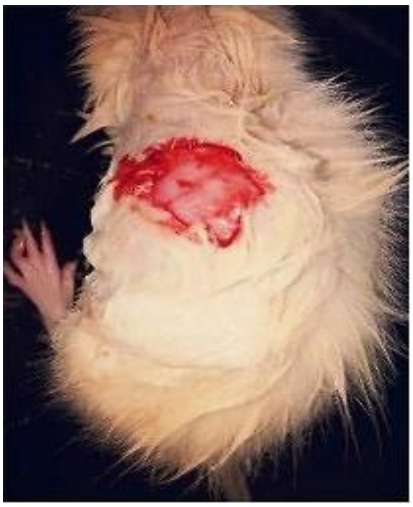

Fig. 2. Wound Creation in Albino Rat

4. RESULTS

4.1 Preliminary Screening of Phytochemicals

4.1.1 Test for alkaloids (Dragendorff's test)

To 2-3 ml extract, add few drops Dragendorff's reagent (solution of potassium bismuth iodide prepared from basic bismuth nitrate [$Bi(NO_3)_3$], tartaric acid, and potassium iodide (KI). Formation of orange brown precipitate indicated the presence of alkaloids (Fig: 3A).

4.1.2 Test for carbohydrate (Molisch test)

The extract was treated with few drops of alcoholic alpha-naphthol. To this 0.2 ml concentrated sulfuric acid was added slowly along the sides of test tube, purple to violet colour ring appeared at junction. This confirmed the presence of carbohydrate in the extract (Fig: 3B).

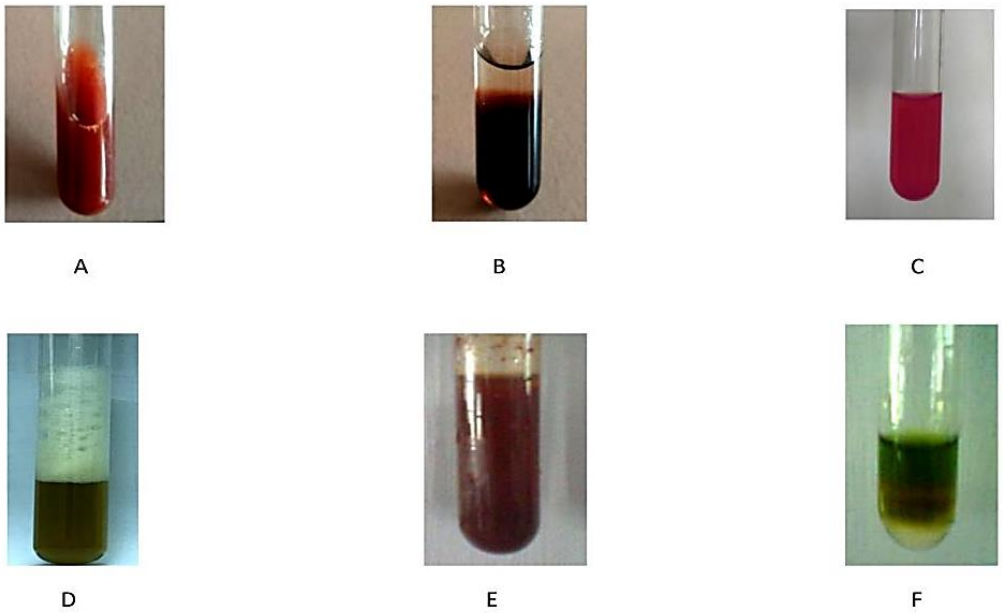

Fig. 3. Preliminary Phytochemical screening of aqueous extract of *Jasminum auriculatum*. A: Test for alkaloids (Dragendorff's test), B: Test for carbohydrate (Molisch test), C: Test for flavonoids, D: Test for saponins (Frothing test), E: Test for steroids and terpenoids (Liebermann-Burchard test), F: Test for tannins and phenolic compounds (5% ferric chloride test)

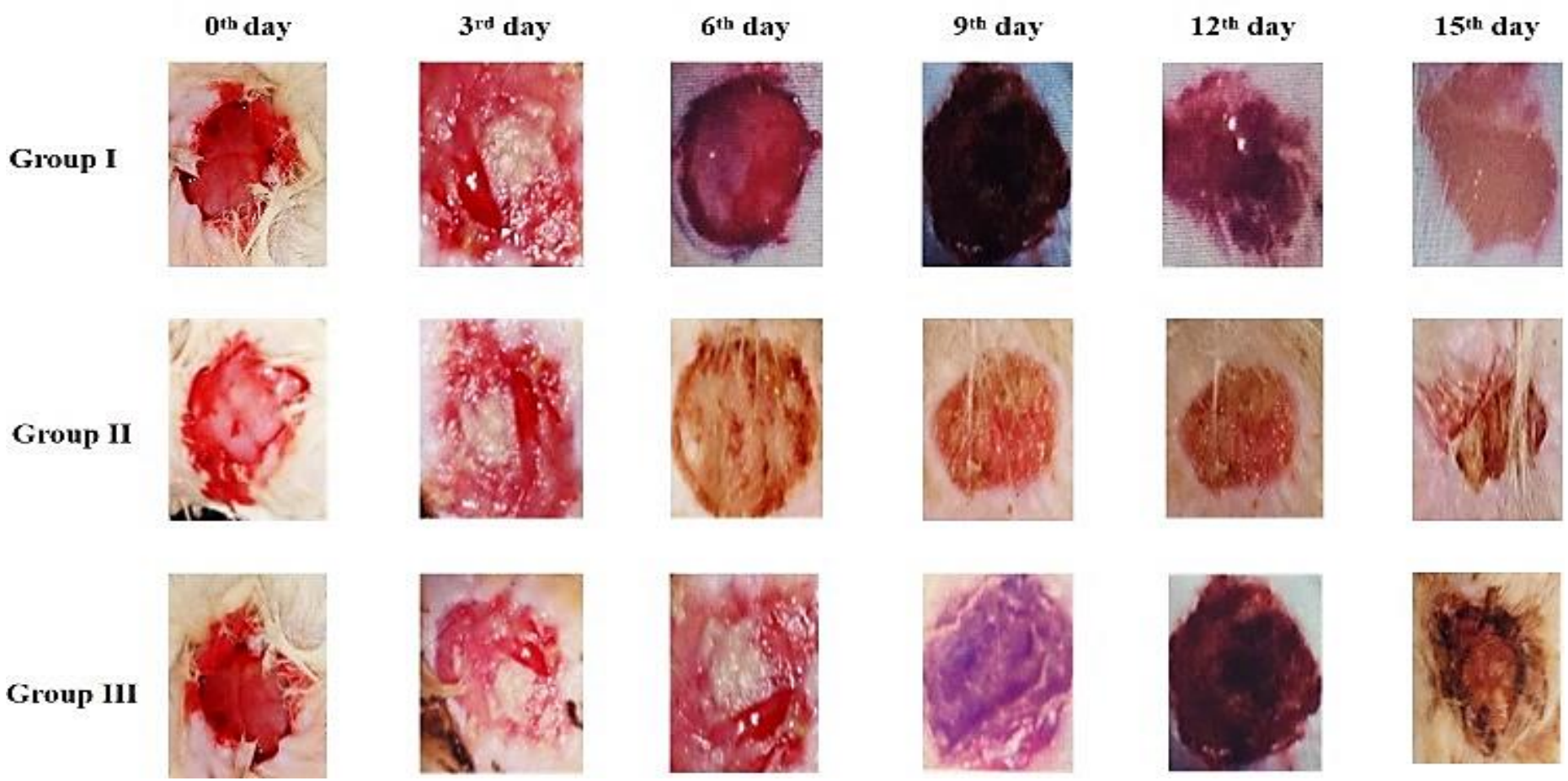

Fig. 4. Depicting reduction in wound area

Table 1. Percentage wound contraction in aqueous extract

DAYS OF TREATMENT → GROUPS ↓	3rd DAY	6th DAY	9th DAY	12th DAY	15th DAY
GROUP I NON-TREATED	6.66 %	13.33 %	20.00 %	26.66 %	33.33 %
GROUP II TREATED WITH SOFRAMYCIN	11.76 %	23.52 %	35.29 %	55.94 %	70.58 %
GROUP III AQUEOUS EXTRACT OINTMENT	6.83 %	15.83 %	35.00 %	55.83 %	61.66 %

4.1.3 Test for flavonoids

In a test tube, a few particles of magnesium metal were added to 2-3 ml of extract, followed by gradual additions of concentrate hydrochloric acid. The formation of magenta color revealed the presence of flavonoids (Fig: 3C).

4.4.4 Test for saponins (frothing test)

In a test tube, 5 ml of extract was quickly agitated with 5 ml of distilled water and warmed. The presence of saponins was determined by the production of stable foam (Fig: 3D).

4.1.5 Test for steroids and terpenoids (Liebermann-burchard test)

Chloroform was combined with 2ml of the extract from the test tube's side, 1-2 mL of acetic anhydride, and 2 drops of concentrated sulfuric acid were added. Because this extract failed to indicate indicators such as the presence of red, blue, and finally green color for the presence of steroids, as well as the presence of pink color for the existence of terpenes. As a result, the test for steroids and terpenes was negative (Fig: 3E).

4.1.6 Test for tannins and phenolic compounds (5% ferric chloride test)

2 mL of extract was mixed with an equal amount of distilled water, and a few drops of ferric chloride ($FeCl_3$) solution were added. The formation of green precipitate indicated the presence of tannins (Fig: 3F).

4.2 Wound Contraction Rate

Wound contraction rate refers to the rate at which a wound or injury reduces in size as the edges of the wound pull closer together. In other words, it is the process by which a wound naturally reduces in size due to the contraction of the surrounding tissues. The wound contraction rate is an important factor in the healing process, as it can help to close the wound and reduce the risk of infection.

To compare the wound contraction rates of three groups: I, II and III (i.e. non-treated group, standard treated group, and group treated with an aqueous extract of *Chromolaena odorata* respectively). On postoperative days 3, 6, 9, 12, and 15, the non-treated group demonstrated a wound contraction rate of 6.66%, 13.33%, 20%, 26.66%, and 33.33%, respectively. On the same days, the standard treated group showed higher wound contraction rates of 11.76%, 23.52%, 35.29%, 55.94%, and 70.58%, respectively. The group treated with the *Chromolaena odorata* extract also showed increasing wound contraction rates of 6.83%, 15.83%, 35%, 55.83%, and 61.66% on postoperative days 3, 6, 9, 12, and 15, respectively (Table 1). Overall, the wound contraction rates were higher in the standard treated and *Chromolaena odorata* treated groups compared to the non-treated group, with the highest rates observed in the standard treated group [10].

5. DISCUSSION

“Wound healing is a sequence of events which consists of coagulation, inflammation, collagenation, wound contraction and epithelialization. While the phase between coagulation to collagenation is intimately inter-linked, the phase of wound contraction and epithelialization are independent to each other and run concurrently” [2]

“In order to evaluate wound healing activity, no single model is adequate to collectively represent the various components of the wound healing process as a whole. Hence, in the present study two different wound models were used to establish the healing potential of successive ethanolic extract of *Chromolaena odorata* on various phases” [4].

“Collagen, the major protein of extracellular matrix, is the component which gives strength, support and integrity to the wound matrix. Breakdown of collagen liberates free hydroxyproline and its peptides. A healing tissue synthesizes collagen, which is a constituent of growing cells. Therefore, measurement of

hydroxyproline can be used as an index for collagen turnover" [8,9].

"The results obtained in the present study proved that the aqueous extract of *Chromolaena odorata* have wound healing efficacy when evaluated by measuring wound contraction using excision wound model" [6]. "Further wound healing property of *Chromolaena odorata* may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that hastens the process of wound healing. From the present study, it is concluded that all of the successive extracts i.e. petroleum ether, chloroform, ethanol and aqueous extracts possess wound healing potential" [10,5]. "Measurement of wound contraction in excision wound model concludes that ethanolic extract is more potent. Detailed study upon this extract based on measurement of wound contraction, lesser epithelialization period, increased tensile strength, increased collagenation, histopathology, antioxidant, antimicrobial and phytochemical analysis supports the idea that successive ethanolic extract has remarkable effects. Further studies with purified constituents are needed to understand the complete mechanism of wound healing potential and constituents responsible for the same activity".

"The preliminary phytochemical analysis revealed the presence of flavonoids, triterpenoids, steroids, alkaloids, saponins, tannins and phenolic compounds in successive aqueous extract. As these agents influence one or more phases of healing process, hence, accelerating it. Flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence, any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the circulation, preventing the cell damage and by promoting the DNA syhthesis" [10].

Flavonoids and Triterpenoids are also known to promote the wound-healing process mainly due to their anti-microbial and free radical scavenging, which seems to be responsible for wound contraction and increased rate of epithelialization.

6. CONCLUSION

The wound healing potential of standard and aqueous extract of *Chromolaena odorata* was studied on Wistar rat excision wound model. The topical application of standard (soframycin) showed a significantly faster wound contraction rate (70.58%) on 15th post-operative day as compared to non-treated group (33.33%). Aqueous extract of *Chromolaena odorata* showed wound contraction rate of (61.66%) on 15th post-operative day as compared to non-treated group (33.33%).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The animal Experiment has been approved by IAEC, SPS, S 'O' A University bearing Regd. No-1171/PO/Re/S/08/CCSEA

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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