

## The Wound Healing Effect of Ointment Formulation Containing *Chromolaena odorata* on Rats

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### Abstract

The methanolic extract of *Chromolaena odorata* (Asteraceae) was screened for wound healing properties. The wound healing effect was determined using the excision wound model on rats. The extract was used to formulate ointments and the formulations compared to penicillin ointment, a standard antibiotic. The results obtained showed a progressive decrease in wound area with time in all the ointments, indicating an efficacy of the herbal formulation in healing the wounds. By the 19<sup>th</sup> day, the ointment containing 10 % w/w of *Chromolaena odorata* methanolic extract showed 100 % healing. The wounds treated with the standard penicillin ointment showed a 95.6 % healing by the 19<sup>th</sup> day, indicating that the plant extract at 10 % w/w had a better wound healing property than the standard antibiotic. The percentage healing in the negative control (petroleum jelly alone) was significantly different ( $p < 0.05$ ) from those of the extract and antibiotic treated groups.

**Key words:** Wound healing, Methanolic extract, *Chromolaena odorata*, Penicillin ointment

### Introduction

The World Health Organisation (WHO) redefined traditional medicine recently as health practices, approaches, knowledges and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well being (Wikipedia, 2007). It comprises of therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern scientific medicine and are still in use today (Evans, 1996). It includes the material world, the sociological environment whether living or dead and the metaphysical forces of the universe (Sofowora, 1986)

The use of medicinal plants has long been explored in folklore and significant successes recorded have led to investigation into medicinal plants with a view of confirming their acclaimed antimicrobial and wound healing properties. One of such plants is *Chromolaena odorata* (Asteraceae), found commonly in American subtropics is very much widespread in West Africa from the coastal fringes of the rainforest to the Southern part of Guinea Savannah (Muniappan, 1991). The juice of the crushed leaves when applied to cuts helps to stop bleeding. Other reported medicinal uses include antidiarrhoeal, antispasmodic, antihypertensive, anti-inflammatory and diuretic effects (Iwu, 1993). Studies on the antimicrobial properties revealed that the chloroform and acetone extracts of the plant exhibited significant *in vitro* antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* (Okigbo, 2005; McFayden, 1990) and *Aspergillus niger* (Ngane, 2006; Iwu, 1993)

The aim of this present work, therefore, is to study the wound healing properties of

*Chromolaena odorata* so as to propose a cheaper and effective alternative in wound dressing.

### Materials and Methods

**Plant materials:** Fresh leaves of *Chromolaena odorata* were collected from Nsukka, Enugu State, Nigeria. They were identified by Mr. P.O. Ugwu of the Department of Botany, University of Nigeria, Nsukka.

**Preparation of extracts:** The fresh leaves were washed with tap water, then cut into small fragments and sun dried. The dried leaves were ground into powder with pestle and mortar and 100 ml of methanol was used to extract 50 g of the material at room temperature for 48 h. The extract was concentrated to dryness *in vacuo*, weighed and the refrigerated until required for use.

**Animal studies:** Twenty healthy rats of both sexes obtained from the animal unit of the Department of Veterinary Medicine, University of Nigeria, Nsukka were used in the evaluation of the wound healing properties of the plant extracts. The animals were kept in five groups of four animals per group. They were kept for a period of 3 weeks before commencement of the wound healing studies. They had free access to feed and clean drinking water during the period of acclimatization and throughout the experimental period.

**Preparation of ointment:** The ointments were prepared according to a standard procedure (Carter, 1987). Varying amounts of the methanolic extract (5 % w/w, 10 % w/w, 15 % w/w) were added to the ointment base and stirred gently and continuously until a homogenous product was obtained. It was allowed to cool to a semi-solid and each of the formulations was packed in a clean ointment jar and labeled.

Table 1: Mean wound area of animals treated post-surgery

Groups	Mean wound area (mm <sup>2</sup> )						
	1 <sup>st</sup> day	4 <sup>th</sup> day	7 <sup>th</sup> day	10 <sup>th</sup> day	13 <sup>th</sup> day	16 <sup>th</sup> day	19 <sup>th</sup> day
A (5 % extract)	16.0±0.0	14.0±0.0	13.0±0.7	7.0±1.1	5.5±1.3	4.0±1.1	3.0±1.1
B (10 % extract)	16.0±0.0	14.0±0.0	10.7±0.3	5.5±1.4	3.3±0.9	1.0±0.6	0.0±0.0
C (15 % extract)	16.0±0.0	14.0±0.0	10.0±1.1	4.3±0.5	2.8±0.3	1.5±0.3	0.5±0.3
D (Penicillin ointment)	16.0±0.0	14.0±0.0	9.7±0.6	4.7±1.3	3.3±1.2	1.3±0.9	0.7±0.7
E (Petroleum jelly)	16.0±0.0	14.0±0.0	12.0±0.6	7.7±0.3	6.3±0.3	4.3±0.3	3.0±0.6

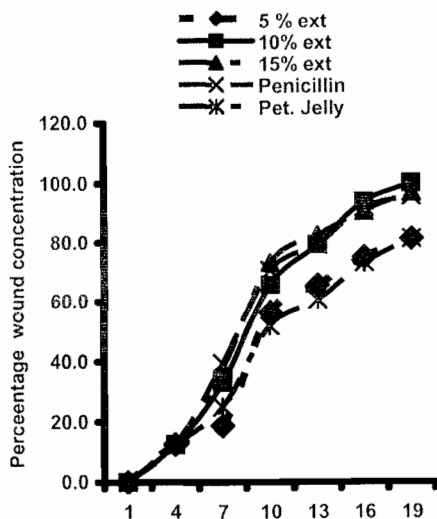


Fig. 1: Effect of ointment formulations containing extracts of *Chromolaena odorata* on percentage wound size contraction in albino rats

**Evaluation of the extract for wound healing properties:**

Wound sites were prepared as described by Dash et al, (2001). Procedures involving animals were conducted in accordance with U.S. guidelines as contained in the NIH guide for the care and use of laboratory animals. Each rat was anaesthetized with diethyl ether and the hair on the skin of the back was clipped. The clipped area was disinfected with 70 % ethanol. A circular incision of 20 mm in diameter was made on the disinfected area of the skin surface and the skin carefully dissected out. The wound area was measured immediately by placing a transparent tracing paper over the wound and tracing it out. The tracing paper was placed on a 1 mm<sup>2</sup> graph sheet and traced out. The squares were counted and the area recorded.

The treatment which started shortly after wound was produced consisted of applying 10 g ointment of 5 % w/w, 10 % w/w and 15 % w/w extract respectively on the wound. Group A animals were treated with 5 % extract; Group B with 10 % extract; Group C with 15 % extract; Group D which

served as the positive control was treated with penicillin ointment and petroleum jelly was applied on the wound of Group E animals (negative control). The extract as well as the penicillin ointment and petroleum jelly were applied topically once daily for 10 days. The wound area of each animal was measured under light diethyl ether anaesthesia on the 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup>, 13<sup>th</sup>, 16<sup>th</sup>, and 19<sup>th</sup> day post-surgery. The percentage wound healing on these days was determined.

The data on percentage wound healing were statistically analyzed using one way analysis of variance (ANOVA). The probability level was 5 %.

**Results and Discussion**

The result of the wound healing effects of the various ointment formulations is presented in Table 1. The result shows that there was significant daily decrease in the wound area throughout the experimental period in all the groups. On the 4<sup>th</sup> day post surgery, all the groups had almost equal mean wound area. This shows that simple ointment which was the base for all the groups formed a protective layer over the wounds and thus did not encourage the growth of microorganisms (Carter, 1987)

There was a sharp reduction in the wound area between the 7<sup>th</sup> and 10<sup>th</sup> day for Group B (containing 10 % extract), Group C (containing 15 % extract) and Group D (penicillin ointment) and this was followed by a gradual decrease on subsequent days. The wound area in Group E (petroleum jelly) also decreased gradually although it was significantly less than those of Group B (10 %), Group C (15 %) and Group D (penicillin ointment). The wound size reduction in Group A was also less than the other concentrations probably because the concentration of the extract was low. This suggests that 5 % of the extract was below the minimum effective concentration for wound healing.

On the 19<sup>th</sup> day post-surgery (Fig. 1), 100 % healing was recorded in Group B extract (containing 10 %) and more than 95 % in Group C (containing 15 %) and Group D (containing penicillin ointment).

This reduction was significantly different from Group E (petroleum jelly group) (81 %) and also Group A (81 %). Overall, the best activity was observed in the formulation containing 10 % *Chromolaena odorata* extract which is probably the optimum concentration. At higher concentrations, there may be decreased diffusion of the extract out of the formulation as observed in Group C. In summary, this study has confirmed the folkloric use of this plant in wound dressing.

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