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# Wound Healing Activity of Ethanolic Extract of Chromolaena Odorata L. on Excision Wound Model In Rats

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**ABSTRACT**

*Chromolaena odorata (L. f.) King and Robinson formerly called as Eupatorium odoratum belongs to the family Asteraceae. C.odorata is a perennial scandent or semi-woody shrub. The aqueous extract and the decoction from leaves of this plant have been used throughout Vietnam for the treatment of soft tissue wounds and burns. The present study was aimed at evaluation of wound healing activity of leaves of C.odorata. Healthy male Wistar albino rats were topically treated with extract formulated in gel (10% and 20% of C.odorata ). Rats of standard groups were treated with 5% w/w povidone-Iodine ointment topically. The percentage of wound contraction was measured. Topical application of C.odorata gel in excision wound model increased percentage of wound contraction. The experimental data revealed that the C. odorata displayed remarkable wound healing and thus supports its traditional use.*

**KEYWORDS : *Chromolaena odorata L.*, wound healing, excision**

## INTRODUCTION

Wound is defined as breaking of cellular and anatomic or functional continuity of skin or mucous membrane and is associated with loss of function. Wound healing is a complex dynamic process involving a number of steps, including induction of inflammatory process, regeneration of parenchyma tissue, migration and proliferation of parenchymal tissue cells, production of EMP's (Extracellular matrix proteins), remodelling of tissue and gaining wound strength. All these are carried out in a sequential manner by wide range of cytokines including growth factors like platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), epidermal growth factor (EGF) and fibroblast growth factor (FGF) [1] .Wide range of substances like tissue extracts [2], vitamins & minerals and a number of plant products [3] have been reported by various workers, to possess, pro-healing effects.

*Chromolaena odorata* (L.*f.*) King and Robinson formerly called as *Eupatorium odoratum* belongs to the tribe of Eupatorieae in the sunflower family *Asteraceae*. *C.odorata* is a perennial scandent or semi-woody shrub. *C.odorata* is very much abundant and widespread in nature. *C.odorata* contains a wide range of secondary metabolites, including flavonoids, terpenoids, and alkaloids. *C.odorata* is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc [4]. *C.odorata* is a highly valuable medicinal plant having different compounds with antioxidant, anti-inflammatory, wound healing and other activities proving the folklore use of this plant by the tribal's [5].Therefore the present study was aimed at evaluation of wound healing activity of ethanol extracts of *C.odorata* leaves.

## MATERIALS AND METHODS

### Plant materials

The samples (leaves) of *C.odorata* used in this study were collected from kodaikanal hills, Tamil Nadu. *C.odorota* was identified based on its physical characteristics. The plant material was washed with distilled water to remove dust and sand. Plant samples were shade dried and manually ground to a fine powder.

### Preparation of extract

The dried powder of *C. odorata* plant was soaked in sufficient volume of 90% ethanol solution for 24 h followed by cold maceration for further 48 h with occasional shaking. The mixture was filtered using muslin cloth followed by removal of excess of solvent by rotatory evaporator. The dried extract was used for the study [6].

### Phytochemical Screening

The powdered leaves of *C.odorata* was subjected to successive extraction with different solvents like petroleum ether, chloroform, acetone,ethanol and water. The dry extracts were subjected to various chemical tests.All the extracts obtained were subjected to various qualitative tests in order to detect the presence of different phytoconstituents [7,8].

### Pharmacological Activity

### Experimental animals

Permission for animal studies from Institutional Animals Ethics Committee (IACE) (Reg. No.688/02/T-CPCSEA) was taken.Healthy male Wistar albino rats weighing 190 - 220gms were procured and were used for the study .They were maintained at 24-28$^0$C, housed individually with free access to food and water. They were fed with standard diet and kept in well-ventilated animal house with alternate dark-light cycle of 12 hrs throughout the studies.

### Induction of wound

An excision wound was inflicted by cutting away approximately 500 mm$^2$ full thickness of shaved skin at a pre-determine area on the anterio dorsal side of the rats under sodium pentobarbital (60 mg/kg., i.p) anesthesia. The entire wound was left open. Animals were closely observed for any infection and those which show signs of infection were separated and excluded from the study and replaced.

### Wound healing activity

Totally 24 animals were divided into 4 groups of 6 animals each. Excision wound was inflicted in all the rats of 4 groups as mentioned above. Group I was served as vehicle control, the wounds were treated with simple ointment base. Group II served as reference control, the wounds were treated with standard povidone iodine ointment. The wounds of animals in group III & IV were treated with topical application of ethanolic extract of *C.odorata* (10% & 20% respectively) by admixing in simple ointment. All the test drugs were applied topically on the wound, twice daily for 21 days.

### Assessment of wound contraction

Wound contraction was monitored by metric measurement of the wound area once on 1$^{st}$, 4$^{th}$, 7$^{th,}$ 14$^{th}$ and 21$^{st}$ day of post wounding days. The wound contraction was measured as a percentage decrease of original wound size of 500 mm$^2$ for each animal of a group.

### Histopathological Study:

The granulation tissues were obtained on day 21 from the test and control group animal for the histological study.

### Statistical Analysis

Results were expressed as mean ± SEM. The data was analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's t test. A P value < 0.05 was considered as significant.

## RESULTS

Phytochemical studies showed that leaf extracts of *C.odorata* contains saponins, quinone, tannins, cardiac glycosides, coumarins, flavonoids, terpenoids, carbohydrates, alkaloids, steroids and beta cyanin (Table 1).

**Table 1 -Phytochemical screening from leaf extracts of *C. odorata* using various solvents**

| Phytochemicals Tested | Leaf Extracts of *Chromolaena odorata* | | | | |
|---|---|---|---|---|---|
| | Aqueous | Ethanol | Chloroform | Petroleum ether | Acetone |
| Tannins | ++ | ++ | + | + | + |
| Saponins | + | + | - | - | - |
| Quinones | + | ++ | + | + | + |
| Terpenoids | + | ++ | + | + | + |
| Steroids | + | ++ | + | + | + |
| Flavonoids | ++ | ++ | + | + | + |
| Phenol | ++ | ++ | + | + | + |
| Alkaloids | + | ++ | - | - | + |
| Glycosides | - | - | - | - | - |
| Cardiac glycosides | + | ++ | - | - | + |
| Coumarins | + | ++ | + | + | - |
| Antho cyanin | - | - | - | - | - |
| Beta cyanin | + | + | + | + | + |

+ Positive, ++ strong positive, - negative

The results of wound healing activity of ethanolic extract of *C.odorata* (10% & 20%) in rats are shown in Table 2 and the percentage wound contraction is shown in Table 3. Wound healing was promoted by the topical applications of ethanolic extract of *C. odorata* (10% & 20%) when compared to control. The wound contraction of reference control and both the doses of C.odorata were 476.97±10.53, 406.22±6.34 & 459.31±8.92 respectively. From the study it was concluded that C.odorata at the dose of 20% showed significant wound healing activity than 10%. The wound healing activity of both the doses of *C.odorata* was comparable with reference control, povidone iodine ointment in excision wound model in rats (Figs.1 & 2).

**Table 2 - Effect of ethanolic extract of *C. odorata* on excision wound in rats**

| Groups | Drug Treatment | Wound Contraction (days) | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 4 | 7 | 14 | 21 |
| I | Control (Simple Ointment) | 15.66 ±0.96 | 42.21 ±1.32 | 95.87 ±2.54 | 176.90 ±7.99 | 232.22 ±12.36 |
| II | Povidone Iodine Ointment | 19.56 ±1.24 | 134.62 ± 5.96*** | 267.74 ±7.75*** | 396.21 ±9.04*** | 476.97 ±10.53*** |
| III | SAMPLE (*C.odorata* 10%) | 17.03 ±1.23 | 96.24 ±6.21** | 195.57 ±6.60** | 297.21 ±7.30*** | 406.22 ±6.34*** |
| IV | SAMPLE (*C.odorata* 20%) | 18.59 ±0.98 | 124.57 ±6.92*** | 249.02 ±5.55*** | 375.62 ±10.54*** | 459.31 ±8.92*** |

Values are in Mean ± SEM; (n = 6)

*P < 0.05, **P < 0.01, *** P < 0.001 Vs Control

**Table 3 - Effect of ethanol extracts *C. odorata* (10% & 20%) on excision wound in rats (% Wound Contraction).**

| Groups | Drug Treatment | % Wound Contraction (days) | | | |
|---|---|---|---|---|---|
| | | 4 | 7 | 14 | 21 |
| I | Control (Simple Ointment) | 31.35 | 35.80 | 44.65 | 48.69 |
| II | Povidone Iodine Ointment | 100 | 100 | 100 | 100 |
| III | SAMPLE (*C.odorata* 10%) | 71.49 | 73.04 | 75.01 | 85.17 |
| IV | SAMPLE (*C.odorata* 20%) | 92.53 | 93.01 | 94.80 | 96.29 |

**Fig 1 Excision wound healing (topically treated) for vehicle and reference controls for different duration**

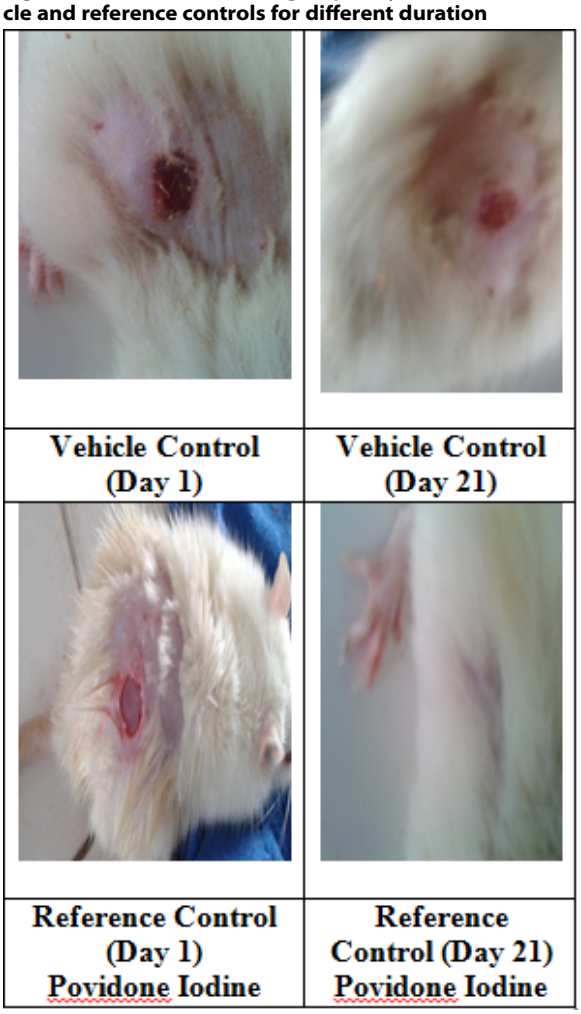
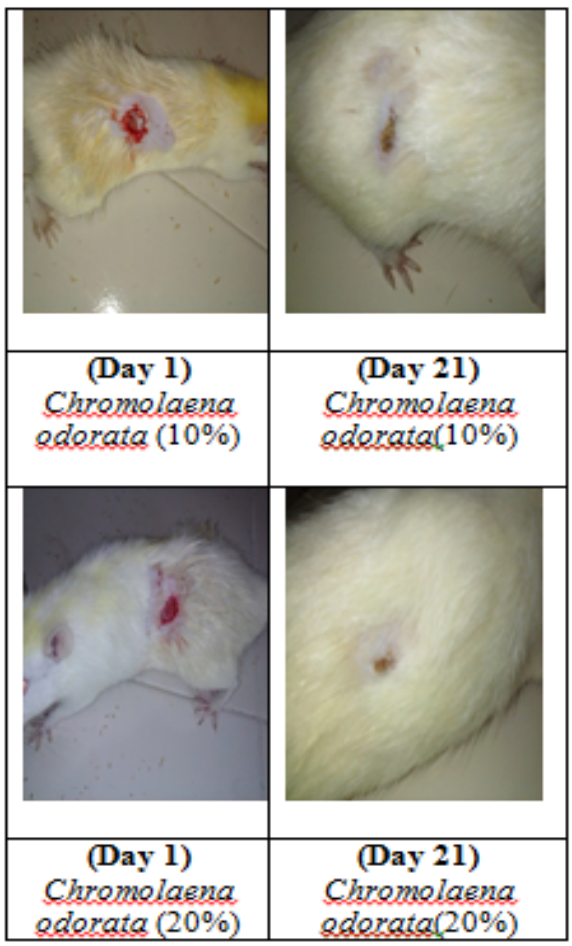


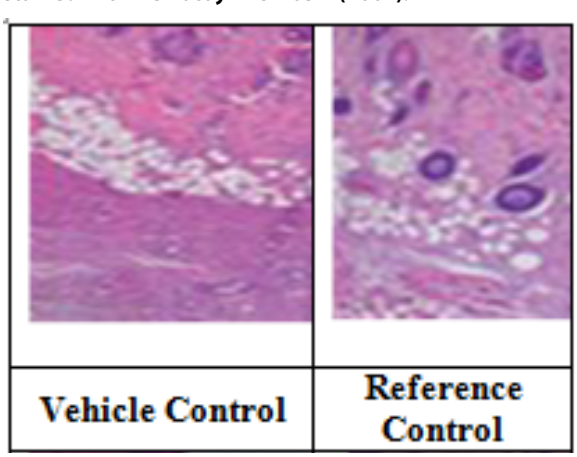
**Fig 2 Excision wound healing (topically treated) for 10 % and 20% plant extract for different duration**

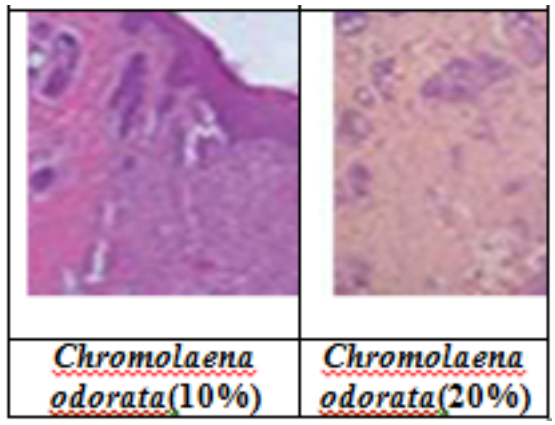



**Histopathology**

Histopathology of excision wound skin at day 21 stained with Hematoylin & Eosin (100x) was shown in Fig 3. The Skin of control rat showed ulceration and edema, and granulation tissue and abundance of mononuclear inflammatory cells. Povidone treated rats showed large amount of granulation tissue, re-epithelization and small number of mononuclear inflammatory cells. *C.odorata* (10%& 20%) treated rats showed moderate healed skin structures with formed and extensive fibrosis and collagen tissue within the dermis.

**Fig 3 Histopathology of excision wound skin at day 21 stained with Hematoylin & Eosin (100x).**




**Discussion**

In the present study, topical application of *C.odorata* extract significantly accelerated wound healing with 20% ointment having the highest percentage wound contraction ability followed by povidine (a commercially sold topical antibiotic agent used in wound dressing) and 10% ointment of *C.odorata*. This may be due to the stimulation of interlukin-8 and inflammatory α- chemokine, which in turn enhances the function of various inflammatory cells, fibroblast and keratinocytes (Udupa *et al*., 2006). Several phytoconstituents like alkaloids [9] and saponins [10] supports wound healing because of their antibacterial, antioxidant and other pharmacological activities. Tannins act as free radical scavengers; triterpenoids promote wound healing due to their astringent and antimicrobial property and saponins due to their antioxidant and antimicrobial activity, which appear to be responsible for wound contraction and elevated rate of epithelialization. Plant Sterols and phenolic compounds are also responsible for wound healing due to free radical-scavenging and antioxidant activity, which are known to reduce lipid peroxidation, by reducing cell necrosis and improving vascularity [11].

Flavonoids have been documented to possess strong antioxidant effect, which is supposed to be one of the most important components of wound healing [12] by increasing the level of antioxidant enzymes in granuloma tissue [13]. Flavonoids reduces lipid peroxidation by improving vascularity in addition to delaying or prevention of onset of cell necrosis. Lipid per oxidation is a crucial process in many types of injuries like burns, infected wounds and skin ulcers. Thus any plant based drugs which inhibits lipid peroxidation is believed to increase strength of collogen fibres, by increasing circulation or by preventing cell damage or by promoting DNA synthesis. Defending the cells against inflammatory mediators may be the possible way in which *C.odorata* promotes wound healing [14]. Free radical scavengers play a vital role in wound healing process by protecting tissues from oxidative damage [15]. Preliminary phytochemical analysis of the leaves of *C.odorata* revealed presence of flavonoids, tannins, sterols, saponins, alkaloids, phenolic compounds and glycosides.

**Conclusion**

The enhanced wound healing may be due to free radical scavenging action and the antibacterial property of the phytoconstituents present in the plant extract. This could be the reason for prohealing activity of *C.odorata*. This enhanced wound contraction effect of *C.odorata* and epitheliazation could possibly be made use of clinically in healing of open wounds.

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