



Effectiveness of a Combined *Chromolaena odorata* L. Leaf and Turmeric Rhizome Ointment on Incision Wound Length in Mice Based on Macroscopic Assessment

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Abstract: This study aimed to determine whether a combination of Kirinyuh leaf (*Chromolaena odorata* L.) and turmeric rhizome extracts affects incision wound length in mice based on macroscopic evaluation. This experimental study employed a randomized post-test-only control group design within a completely randomized design (CRD). A total of 25 male mice were divided into five treatment groups. Combination ointments containing Kirinyuh leaf and turmeric rhizome extracts at a 3:1 ratio were prepared at concentrations of 2.5%, 5%, and 10%. An incision measuring 1 mm in length was created on the dorsal region of each mouse. The wounds were treated topically twice daily with the extract ointment, and macroscopic observations were performed daily from day 0 to day 14. The results showed no significant differences among the groups on day 7. By day 14, most groups had achieved wound closure. The P2 group treated with the 2.5% combination ointment exhibited the lowest mean wound length (0.426 ± 0.073 mm), whereas the negative control group showed the highest mean wound length (1.296 ± 0.804 mm). These findings indicate that the combination of Kirinyuh leaf (*Chromolaena odorata* L.) and turmeric rhizome extracts may contribute to the healing of incision wounds in mice.

Keywords: Kirinyuh leaf; turmeric rhizome; wound healing; mice

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INTRODUCTION

Indonesia is a tropical country with abundant natural resources and a high diversity of plant species that have potential applications in human and animal health. Many of these plants contain bioactive compounds that may contribute to disease prevention and wound management (Nurhajanah et al., 2020). Herbal remedies are also relatively accessible because medicinal plants can often be obtained from the surrounding environment. Among the traditional medicinal plants commonly recognized and used by local communities are Kirinyuh (*Chromolaena odorata* L.) and turmeric rhizome (Arisonya et al., 2014).

Kirinyuh (*Chromolaena odorata* L.) is a fast-growing wild plant that is frequently regarded as an invasive weed. Despite its widespread distribution, the plant has considerable medicinal potential and has traditionally been used for wound treatment and several other health conditions (Sagala, 2009). Its leaves contain various bioactive compounds, including tannins, phenols, alkaloids, terpenoids, flavonoids, saponins, and steroids (Vital & Rivera, 2009). More recent reviews have also reported that *C. odorata* contains flavonoids, phenolics, tannins, alkaloids, saponins, and terpenoids that contribute to its antimicrobial, anti-inflammatory, antioxidant, hemostatic, and wound-healing activities (Sirinthipaporn & Jiraungkoorskul, 2017; Olawale et al., 2022).

Experimental evidence further supports the wound-healing potential of *C. odorata*. Abdul Latif et al. (2024) demonstrated that a *C. odorata*-containing

transdermal formulation significantly enhanced wound contraction and was associated with increased collagen deposition and faster granulation tissue formation in an in vivo rat wound model. These findings provide additional biological support for investigating *C. odorata* as a component of topical wound-healing preparations.

Turmeric (*Curcuma longa* L.) is a rhizomatous plant that has long been used in traditional medicine for the management of digestive disorders, inflammation, wounds, infections, injuries, arthritis, and menstrual disorders. Its functional properties are largely attributed to curcuminoids, particularly curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Hartati, 2013). Curcumin has attracted particular attention in wound-healing research because of its anti-inflammatory and antioxidant properties. It has also been reported to promote collagen deposition, granulation tissue formation, and wound contraction, although its therapeutic application may be limited by poor aqueous solubility and bioavailability (Kumari et al., 2022).

A wound is characterized by disruption of the continuity of the skin epithelium and may result from sharp or blunt trauma, temperature changes, chemical exposure, explosions, or animal bites. Wounds may cause pain, discomfort, and tissue damage and may interfere with daily activities. Without appropriate treatment, the healing process may be prolonged (Wallace et al., 2022). Cutaneous wound healing is a complex and highly coordinated biological process involving overlapping phases of hemostasis and inflammation, proliferation, and tissue remodeling. These phases involve interactions among inflammatory cells, keratinocytes, fibroblasts, endothelial cells, extracellular matrix components, cytokines, and growth factors (Almadani et al., 2021; Gushiken et al., 2021).

The progression of wound healing can be influenced by several systemic and local factors, including age, anemia, underlying disease, vascularization, nutritional status, obesity, medication use, personal hygiene, and stress (Nurani et al., 2015). Disruption of the normal inflammatory, proliferative, or remodeling response may delay tissue repair and contribute to impaired or chronic wound healing (Almadani et al., 2021).

Conventional wound management commonly involves antiseptic agents such as povidone iodine. However, prolonged use may cause tissue irritation and potentially interfere with epithelialization, thereby encouraging the exploration of alternative therapies that are effective and biologically compatible. One approach that has received increasing attention is the use of plant-derived compounds with antibacterial, anti-inflammatory, antioxidant, and tissue-regenerative activities (Purnama et al., 2017). Natural compounds that modulate inflammation, oxidative stress, angiogenesis, fibroblast activity, collagen deposition, and re-epithelialization have therefore become important candidates for the development of topical wound-healing therapies (Gushiken et al., 2021).

Kirinyuh leaves contain phytochemicals with antibacterial, antioxidant, and anti-inflammatory activities, whereas turmeric rhizomes provide curcuminoids with well-documented antioxidant and anti-inflammatory properties. Because these plants contain different but potentially complementary bioactive constituents, their combined application may influence several biological processes involved in wound repair. However, the presence of complementary pharmacological activities alone does not establish a synergistic effect; such an interaction must be demonstrated experimentally. Evidence from previous studies supports the wound-healing activity of *C. odorata* and curcumin individually, but studies evaluating their combined application in a topical ointment remain limited.

Therefore, this study aimed to evaluate the effectiveness of an ointment containing a combination of Kirinyuh leaf (*Chromolaena odorata* L.) and turmeric rhizome (*Curcuma longa* L.) extracts in promoting incision wound healing in mice based on macroscopic observations. The findings are expected to provide preliminary evidence regarding the potential of this combination as a herbal topical formulation for wound management.

METHOD

Study Design

This study was conducted as an experimental study using a randomized post-test-only control group design with laboratory animals as the experimental subjects. The experiment was arranged using a completely randomized design (CRD).

Materials and Equipment

The equipment used in this study included 1-mL syringes, mouse cages, surgical scalpels, a digital balance, stationery, a blender, a food dehydrator, a distillation apparatus, a vernier caliper, and gloves. The materials included Kirinyuh leaves (*Chromolaena odorata* L.), turmeric rhizomes, male mice, alcohol swabs, lidocaine as a local anesthetic, distilled water, povidone iodine, and white petrolatum (*Vaselineum album*).

Experimental Animals and Treatment Groups

Healthy male mice weighing 20–30 g were used as experimental animals. The animals were provided with standard feed and drinking water *ad libitum* and maintained under normal environmental conditions at 18–25 °C. A total of 25 mice were used and divided into five experimental groups, with five mice in each group. The treatment allocation is presented in Table 1.

Table 1. Experimental design for the wound-healing test using Kirinyuh leaf and turmeric rhizome extract ointment

Group	Number of Animals	Treatment
P0	5 mice	Incision wound without treatment, serving as the negative control
P1	5 mice	Incision wound treated with povidone iodine, serving as the positive control
P2	5 mice	Incision wound treated topically with a combination ointment of Kirinyuh leaf (<i>Chromolaena odorata</i> L.) and turmeric rhizome extracts at a concentration of 2.5%
P3	5 mice	Incision wound treated topically with a combination ointment of Kirinyuh leaf (<i>Chromolaena odorata</i> L.) and turmeric rhizome extracts at a concentration of 5%
P4	5 mice	Incision wound treated topically with a combination ointment of Kirinyuh leaf (<i>Chromolaena odorata</i> L.) and turmeric rhizome extracts at a concentration of 10%

Induction and Treatment of Incision Wounds

Before treatment, the mice were acclimatized for one week. Incision wounds were created after the animals had been anesthetized with lidocaine at a dose of 0.3 mL/kg body weight to minimize pain during wound induction. After adequate anesthesia had been achieved, the fur surrounding the intended wound area was shaved. The dorsal skin was then cleaned with 70% alcohol and iodine before incision.

A 1-cm-long incision was created on the dorsal skin using a surgical scalpel, and the wound length was measured using a vernier caliper. The incision was made at the

epidermal layer. The Kirinyuh leaf and turmeric rhizome extract ointment was applied topically twice daily, in the morning and afternoon. Wound healing was monitored by measuring the incision length using a vernier caliper from the first day of wound induction until day 14. The macroscopic parameter evaluated during the observation period was incision wound length.

Preparation of Kirinyuh Leaf and Turmeric Rhizome Extracts

Kirinyuh leaves (*Chromolaena odorata* L.) and turmeric rhizomes were cleaned and washed under running water, drained, cut into smaller pieces, and dried using a food dehydrator. After drying, the plant materials were ground using a blender to obtain Kirinyuh leaf and turmeric rhizome powders.

The powdered plant materials were extracted by maceration in distilled water for 3 × 24 h. The resulting extracts were subsequently concentrated using an evaporator at 50 °C in a water bath until thick extracts were obtained. Kirinyuh leaf and turmeric rhizome extracts were then combined at a ratio of 3:1. The combined extract was subsequently incorporated into white petrolatum as the ointment base. Three ointment concentrations were used in the study: 2.5%, 5%, and 10%.

Preparation of the Ointment Formulations

The 2.5% ointment formulation was prepared by mixing 1.25 g of the combined Kirinyuh leaf and turmeric rhizome extracts with 9.75 g of white petrolatum. The 5% formulation was prepared by mixing 2.5 g of the combined extracts with 9.5 g of white petrolatum, whereas the 10% formulation was prepared by mixing 5 g of the combined extract preparation with 9 g of white petrolatum. Each ointment formulation was placed in a tightly closed sterile container until use.

Data Analysis

Wound-length measurement data were tabulated and statistically analyzed using SPSS. Analysis of variance (ANOVA) was used to evaluate differences among the experimental groups. Duncan's post hoc test was subsequently applied to identify differences among the treatment groups.

RESULTS AND DISCUSSION

Macroscopic evaluation of incision wound healing was performed in 25 mice allocated to five experimental groups. The treatment groups received ointments containing a combination of Kirinyuh leaf (*Chromolaena odorata* L.) and turmeric rhizome extracts at concentrations of 2.5%, 5%, and 10%, whereas the control groups consisted of an untreated negative control (P0) and a povidone iodine-treated positive control (P1). On day 1, wounds in all groups showed swelling and exudate formation. These macroscopic changes were consistent with the early inflammatory phase of wound healing, which is characterized by classical signs of inflammation, including calor (heat), dolor (pain), rubor (redness), tumor (swelling), and functio laesa (impaired function).

In the negative control group (P0), incision wound healing required approximately 13 days, and the wound length measured 6.4 mm on day 7. In the positive control group (P1), treated with povidone iodine, the wound length was 5.8 mm on day 7, with wound closure reported by day 14. In the P2 group, treated with the 2.5% combination ointment, the wound length was 4.5 mm on day 7, and wound closure was reported by day 14. The P3 group, treated with the 5% combination ointment, also showed a wound length of 4.5 mm on day 7 and exhibited the fastest healing response, requiring approximately 9 days. In the P4 group, treated with the 10% combination ointment, the wound length was 5.9 mm on day 7, with wound closure reported by day 14.

Table 2. Mean incision wound length in mice

No.	Treatment	Mean $\pm$ SD
1	P0	1.2960 ^b $\pm$ 80379
2	P1	0.5480 ^a $\pm$ 0.11862
3	P2	0.4260 ^a $\pm$ 0.07335
4	P3	0.4460 ^a $\pm$ 0.15837
5	P4	0.9940 ^{ab} $\pm$ 0.86019

As shown in Table 2, the highest mean incision wound length was observed in the P0 group, whereas the lowest mean wound length was recorded in the P2 group. Significant differences were reported between P0 and P1, P0 and P2, and P0 and P3. In contrast, no significant differences were reported between P0 and P4, P1 and P2, or P2 and P3. The post hoc grouping therefore indicates that the 2.5% and 5% combination ointments produced lower mean wound lengths than the untreated control and responses comparable to the positive control.

**Figure 1.** Incision wound length in mice in groups P0, P1, P2, P3, and P4

Macroscopic observations further demonstrated differences in the progression of wound closure among the experimental groups. The P3 group showed the most rapid apparent wound closure compared with the other groups, while wounds in the P2 and P4 groups also showed faster healing progression than those in the untreated P0 group. Figure 4.1 illustrates the macroscopic wound condition in each group. In the untreated negative control group (P0), the wound remained visible on day 14, with a length of approximately 1.3 mm. In the povidone iodine-treated group (P1), the wound measured approximately 3.7 mm on day 10 and subsequently closed by day 14. In the P2 group treated with the 2.5% combination ointment, the wound measured approximately 2.0 mm on day 11, with closure observed on day 12. In the P3 group treated with the 5% combination ointment, the wound measured approximately 3.7 mm on day 8, with closure observed on day 10. In the P4 group treated with the 10% combination ointment, the wound measured approximately 2.5 mm on day 12, with closure observed on day 13.

Visible signs of healing in the groups treated with the Kirinyuh leaf and turmeric rhizome extract ointments became increasingly apparent between days 9 and 11. During this period, crust formation was observed, the wound surface became progressively drier, and the crust gradually detached from the wound margins. These macroscopic changes were accompanied by progressive wound contraction and a reduction in wound length. This period is consistent with the proliferative phase of wound healing, during which fibroblasts migrate into the wound bed, synthesize extracellular matrix components, and contribute to collagen deposition. Some fibroblasts differentiate into myofibroblasts, which generate contractile forces that approximate the wound margins and contribute to wound contraction (Almadani et al., 2021; Gushiken et al., 2021).

The early inflammatory response followed by progressive wound contraction observed in this study is consistent with the normal biological sequence of tissue repair. Wound healing is a dynamic and highly coordinated process involving overlapping phases of hemostasis, inflammation, proliferation, and remodeling. Following tissue injury, platelet aggregation and fibrin-clot formation contribute to hemostasis. Neutrophils and macrophages subsequently migrate to the wound site, where they remove microorganisms, foreign material, and damaged cells and participate in the release of cytokines and growth factors that regulate subsequent tissue repair. Growth factors such as Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor beta (TGF- β), and Vascular Endothelial Growth Factor (VEGF) contribute to fibroblast and keratinocyte activity, angiogenesis, extracellular matrix formation, and re-epithelialization (Winarsih et al., 2009; Gushiken et al., 2021).

The favorable wound-healing response observed in the treatment groups may be related to bioactive compounds present in *C. odorata*. Kirinyuh leaf extract contains several phytochemical constituents, including tannins, phenolic compounds, flavonoids, saponins, steroids, and terpenoids. Its essential oil has also been reported to contain α -pinene, cadinene, camphor, limonene, β -caryophyllene, and cadinol isomers (Benjamin, 2011). More recent evidence indicates that *C. odorata* possesses antioxidant, antimicrobial, anti-inflammatory, and wound-healing activities that may be relevant to tissue repair (Olawale et al., 2022).

Flavonoids and other phenolic compounds may contribute to wound healing by modulating inflammatory responses and limiting excessive oxidative stress, whereas saponins have been associated with antimicrobial activity and processes related to tissue repair and collagen formation (Yenti, 2011). These biological activities may support the transition from the inflammatory phase to the proliferative phase of healing. However, because the present study did not quantify individual phytochemical constituents or molecular markers, the contribution of specific compounds cannot be determined from the current findings.

Recent in vivo findings also support the wound-healing potential of *C. odorata*. Abdul Latif et al. (2024) demonstrated that a topical transdermal formulation containing *C. odorata* enhanced wound healing in rats and was associated with improved wound contraction and tissue-repair characteristics. Although their formulation and experimental wound model differed from those used in the present study, their findings strengthen the biological plausibility of the favorable macroscopic response observed following topical treatment with a *C. odorata*-containing preparation.

Turmeric rhizome may also have contributed to the reduction in wound length observed in the present study. Turmeric contains curcuminoids, particularly curcumin, which are widely recognized for their antioxidant and anti-inflammatory properties.

Wientarsih et al. (2012) previously reported wound-healing activity associated with turmeric extract, while Mohanty & Sahoo (2017) described the potential application of curcumin in topical wound-healing formulations. More recent evidence suggests that curcumin can modulate inflammatory mediators and oxidative stress and may support fibroblast activity, granulation tissue formation, collagen deposition, and re-epithelialization (Kumari et al., 2022). These properties provide a plausible biological rationale for the inclusion of turmeric rhizome extract in the combination ointment.

The combination of *C. odorata* and turmeric rhizome extracts may therefore provide complementary biological activities during wound healing. *C. odorata* has been associated with antimicrobial, antioxidant, anti-inflammatory, and tissue-repair effects, whereas turmeric-derived curcuminoids are particularly recognized for their antioxidant and anti-inflammatory activities. In general, plant-derived topical preparations may influence wound repair by modulating inflammation, oxidative stress, fibroblast proliferation, collagen deposition, angiogenesis, and epithelial repair (Herman, 2023). Nevertheless, the present study did not include treatment groups receiving Kirinyuh leaf extract or turmeric rhizome extract individually. Therefore, the current findings do not establish whether the two extracts acted synergistically, additively, or independently. A true synergistic effect would need to be demonstrated by directly comparing the individual extracts with their combination under identical experimental conditions.

An important finding of the present study was that increasing the ointment concentration did not consistently result in a greater wound-healing response. The 2.5% formulation showed the lowest mean wound length, whereas the 5% formulation showed the fastest apparent wound closure. In contrast, the 10% formulation did not produce a proportionally greater response. This pattern suggests that the relationship between extract concentration and wound-healing response may not be strictly linear. Differences in extract solubility, diffusion from the ointment base, viscosity, local availability of bioactive compounds, or interactions among phytochemical constituents could potentially influence topical effectiveness. However, these formulation characteristics were not evaluated in the present study; therefore, the mechanisms underlying this concentration-response pattern remain uncertain.

The finding that lower-to-moderate concentrations showed favorable responses is noteworthy because a higher concentration of a herbal extract does not necessarily result in greater biological activity. The effectiveness of a topical formulation depends not only on the amount of extract present but also on the release and availability of its active compounds at the wound surface. Thus, further formulation and pharmacological studies are needed to determine the concentration that provides the most favorable balance between efficacy, stability, and safety.

The findings of this study should also be interpreted in light of several limitations. First, wound healing was evaluated primarily through macroscopic measurements of wound length; histopathological parameters such as inflammatory-cell infiltration, fibroblast density, collagen deposition, angiogenesis, and re-epithelialization were not evaluated. Second, the absence of groups treated with Kirinyuh leaf extract or turmeric rhizome extract alone limits conclusions regarding additive or synergistic effects. Third, each group consisted of only five mice, which may limit statistical power. Finally, the physicochemical characteristics and detailed phytochemical composition of the ointment formulations were not determined. Consequently, the cellular and molecular mechanisms discussed above should be regarded as plausible explanations based on previous literature rather than mechanisms directly demonstrated by this study.

Despite these limitations, the present findings provide preliminary evidence that topical application of an ointment containing a combination of Kirinyuh leaf and turmeric rhizome extracts may support incision wound healing in mice. The lower mean wound length observed in the 2.5% group and the relatively rapid wound closure observed in the 5% group indicate that lower-to-moderate concentrations warrant further investigation. Future studies should include histopathological assessment, quantitative evaluation of collagen and inflammatory markers, characterization of the ointment formulation, toxicity testing, and direct comparison between individual extracts and their combination to clarify the biological basis of the observed wound-healing response.

CONCLUSION

The combination ointment containing Kirinyuh leaf (*Chromolaena odorata* L.) and turmeric rhizome (*Curcuma longa* L.) extracts showed a favorable effect on incision wound healing in mice based on macroscopic observations. The 2.5% treatment group exhibited the lowest mean wound length at the end of the observation period, whereas the 5% treatment group showed the fastest apparent wound closure. These findings indicate that lower-to-moderate concentrations of the combination ointment may support wound healing. However, the response was not strictly concentration-dependent, and the present study does not establish a synergistic effect between the two extracts. Further studies using histopathological assessment, broader concentration ranges, and direct comparison with single-extract treatments are needed to confirm the effectiveness and underlying mechanisms of the combination.

RECOMMENDATION

Further studies are needed to evaluate the effectiveness of the combined Kirinyuh leaf (*Chromolaena odorata* L.) and turmeric rhizome ointment using microscopic and histopathological assessments of wound healing. Additional research should also compare different proportions of Kirinyuh leaf and turmeric rhizome extracts and assess their toxicity profiles to provide a clearer understanding of the activity and safety of the combination in promoting wound healing.

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