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Exploration of the Wound Healing Activity of Ethanol Extract-Based Ointment from Curry Leaves on the Wound Healing Process

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Abstract: Indonesian society widely utilizes traditional medicinal plants, including curry leaves (*Murraya koenigii* L.), which contain alkaloids, tannins, flavonoids, saponins, steroids, and triterpenoids known to possess antioxidant and anti-inflammatory properties. This study aims to formulate curry leaf ethanol extract ointments as wound healing agents and to determine the most effective concentration in accelerating incision wound recovery. Five treatments were prepared: ointment base (negative control), Betadine 10% (positive control), and curry leaf ethanol extract ointments at concentrations of 5%, 10%, and 15%. The ointments were assessed for physical properties and tested on male white rats. The results showed that all formulations met the organoleptic, stability, homogeneity, pH, adhesion, and spreadability requirements. Quantitative wound-healing analysis demonstrated that the 15% extract ointment produced the highest healing percentage, achieving 56.4% on day 5, 70.9% on day 8, and 100% by day 14, outperforming Betadine 10% (80% by day 14). One-way ANOVA revealed significant differences among treatment groups ($p < 0.05$), with the 15% formulation showing the most effective therapeutic response. These findings indicate that curry leaf ethanol extract ointment, particularly at a 15% concentration, has strong potential as a topical Wound Healing agent.

Keywords: exploration; curry-leaves; wound-healing; ethanol-extract; ointment-formulation.

1. INTRODUCTION

Indonesia, as a tropical country, has a rich variety of flora, which is used for medicinal purposes, as protective plants, and as a source of food. One of the most commonly used medicinal plants is curry leaves (*Murraya koenigii* L.). These leaves are known to contain secondary metabolites that have potential biological activity, particularly as antioxidants, [1]. Other studies show that ethanol:water (1:1) extracts from curry leaves have high antioxidant activity because they belong to the polyphenol compound group [2]. The results of research by Trisia et al. (2018) also revealed that curry leaves contain various bioactive compounds, including saponins, terpenoids, lutein, phenols, steroids, flavonoids, and carbazole alkaloids[3].

The curry plant, known by its scientific name *Murraya koenigii* (L.), has different names in various regions of Indonesia, such as temurui (Aceh), sicerek (Minangkabau), and ki becetah (Sunda). Meanwhile, in several other countries, it is called curry (English), garupillai (Malaysia), kerriebladeren (Dutch), feuilles de cari (French), curryblätter (German), fogli di cari (Italian), and hoja de curry (Spanish) [4]. According to Maulina et al. (2023), a number of studies have shown that the carbazole alkaloids in curry leaves have biological activity as anticancer agents and are antimicrobial against both gram-positive and gram-negative bacteria [5]. In addition, curry leaves also contain various important nutrients such as water, protein, fat, vitamin A, vitamin C, potassium, calcium, fiber, copper, iron, and other nutrients. Biologically, this plant is known to be beneficial as an antidiabetic, aiding in weight loss, maintaining gut health, regulating cholesterol levels, supporting wound healing, and acting as an anti-inflammatory.

Curry leaves boiled with coconut oil produce a residue that is often used as a hair tonic, serving to maintain natural hair health while stimulating growth. In traditional medicine, this plant is used as a remedy for diarrhea, fever reducer, antifungal, anti-inflammatory, pain reliever, and antiemetic [6]. Other studies have shown that curry leaf extract has antimicrobial activity, particularly in inhibiting the growth of *Pseudomonas aeruginosa*. Test results showed the highest inhibitory activity at a concentration of 6% with an inhibition zone of 9 mm [7].

Extraction itself is a method of separating a substance from its mixture with the help of a solvent. The solvent used must be able to extract the target compound without dissolving other unwanted components. After the extraction process, the remaining solvent is generally evaporated to obtain a powder mass which is then further processed according to established standards [8].

The wound healing process in damaged body tissue occurs in three main phases, namely inflammation, proliferation, and remodelling or maturation [9]. A wound is a disturbance to the normal condition of the skin characterized by the loss or damage of tissue. Based on the cause, wounds are divided into two types, namely open wounds and closed wounds. One type of open wound is a laceration, which is a tear in the skin and tissue caused by sharp objects such as kitchen knives, broken glass, or zinc [10], [11]. Lacerations are among the most common types of wounds experienced in everyday life [12].

The healing of perineal wounds, for example, is characterized by the formation of new tissue that closes the wound within 3–7 days after delivery [13]. In general, wound healing is a complex and dynamic physiological process involving a series of interactions between cells, chemical mediators, and vascular responses. These three components are interconnected in forming the normal wound healing mechanism. This process can occur naturally or with the help of chemical substances such as drugs or ointments that accelerate tissue recovery [14].

Wound healing occurs through three main mechanisms, namely contraction, epithelialization, and connective tissue accumulation. In this process, damaged or dead tissue is replaced with new, healthy tissue through the mechanism of regeneration. A wound is considered healed when the skin surface is restored and tissue strength returns to normal. Therefore, wound healing is very important to prevent the risk of infection, which can worsen the condition of the wound [15].

In the treatment of incision wounds, topical herbal ingredients are often used because they can accelerate the healing process, especially in the contraction phase of the wound. Topical application allows more active compounds to accumulate in the wound area. One of the commonly used topical preparations is ointment. Ointment is a semi-solid preparation for external use, in which the medicinal ingredients are dissolved or dispersed homogeneously in a suitable base [16].

The ointment base functions as a carrier for the active ingredients, which are generally divided into four types, namely hydrocarbon bases, absorbable bases, water-washable bases, and water-soluble bases. Each base has its own characteristics in releasing the active ingredient. Hydrocarbon bases, for example, are oily like white petrolatum, with high occlusive power that prolongs the contact of the drug with the skin. These bases are difficult to clean, do not dry out, and can last a long time, so they are often used as emollients [16].

Several previous studies have reported the wound healing potential of various medicinal plants formulated into topical preparations, such as *Crescentia cujete* leaves and *Anredera cordifolia* extract; however, studies utilizing curry leaves (*Murraya koenigii* L.) specifically as a wound healing ointment remain limited. Although curry leaves are well known to contain bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial activities, their effectiveness in topical form and the optimal concentration required for incision wound healing have not yet been clearly established. Therefore, this study was conducted to formulate curry leaf ethanol extract into ointment dosage forms and to evaluate their wound healing activity in an incision wound model in male white rats (*Rattus norvegicus*). Furthermore, this research aims to determine the most effective concentration capable of accelerating the wound healing process compared to negative and positive control treatments. The findings of this study are expected to provide scientific evidence supporting the potential use of curry leaf extract as a natural wound healing agent. This release process is influenced by physiological factors of the body as well as the chemical-physical properties of the ointment itself [17].

2. MATERIALS AND METHODS

The research series included plant sampling, ethanol extract preparation, phytochemical screening, ointment formulation, ointment preparation evaluation, test animal preparation, testing on test animals, and measurement of wound healing effects. All research activities were carried out at the STIKes Indah Medan Pharmaceutical Research Laboratory, located at Jalan Saudara Ujung No. 110, Simpang Limun, Medan.

2.1. Materials

The equipment used in this study included laboratory glassware, a blender (Miyako), porcelain dishes, funnels, test animal cages, 500 ml round-bottom flasks, mortars and pestles, pH meters, surgical knives (GEA), ball coolers, rotary evaporators, connecting tubes, water receiving tubes, analytical scales, and digital scales (OHAUS).

The ointment base used for the negative control (Blank) consisted of a hydrophobic base formulated using white petrolatum, adeps lanae, cetyl alcohol, cera alba, and liquid paraffin. The composition of the ointment base per 100 g preparation was as follows: white petrolatum 70%, adeps lanae 10%, cera alba 10%, cetyl alcohol 5%, and liquid paraffin 5%. This base served as the standard formulation to ensure consistency and facilitate comparison with the extract-containing ointments.

2.2. Methods

The effectiveness test of the curry leaf (*Murraya koenigii* L.) ethanol extract ointment was conducted using male white rats (*Rattus norvegicus*) as experimental animals. A total of 25 rats were used, divided into five treatment groups (n = 5 per group): negative control (ointment base), positive control (Betadine 10%), and ethanol extract ointments at concentrations of 5%, 10%, and 15%. The rats used were healthy males aged 2–3 months, with an average body weight of 180–220 grams. The animals were obtained from a certified local breeding supplier.

Before the experiment, all rats were allowed to acclimatize for 7 days under standard laboratory conditions with a temperature of 22–25°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. During the acclimatization and experimental period, the rats were housed in polypropylene cages with rice-husk bedding, given ad libitum access to pellet feed and drinking water. All procedures in this study followed the institutional guidelines for the care and use of laboratory animals and were approved by the Research Ethics Committee of STIKes Indah Medan.

2.3. Sampling

Research samples were obtained through purposive sampling, which is sampling without comparing with plants from other locations.

2.4. Simplisia Preparation Procedure

The collected curry leaves were first wet sorted to separate the leaves from other parts of the plant, dirt, and other foreign materials. Next, the leaves were weighed at ±20 kg, washed with running tap water until clean, then drained and dried in direct sunlight. The drying process is carried out until the leaves are easily crushed. Once dry, dry sorting is carried out to ensure that no dirt remains during the drying process. The simplisia is then weighed again, ground using a blender, and stored in plastic containers at room temperature [18].

2.5. Production of Curry Leaf Ethanol Extract

The curry leaf ethanol extract obtained from the maceration process was formulated into ointment preparations at concentrations of 5%, 10%, and 15%. The ointment base used was a hydrophobic (oleaginous) base composed of white petrolatum, adeps lanae, cetyl alcohol, cera alba, and liquid paraffin. The complete formulation is shown in Table 1.

Table 1. Formulation of Curry Leaf Ethanol Extract Ointments

Ingredients	Blank (%)	EEDC 5%	EEDC 10%	EEDC 15%
Curry leaf ethanol extract	0	5	10	15
White petrolatum	70	65	60	55
Adeps lanae	10	10	10	10
Cetyl alcohol	5	5	5	5
Cera alba	10	10	10	10
Liquid paraffin	5	5	5	5
Total (%)	100	100	100	100

3. RESULTS AND DISCUSSION

3.1. Results of Simplisia Characteristic Examination

The characteristics of curry leaves were examined through macroscopic and microscopic observation, as well as moisture content determination.

3.2. Macroscopic

Macroscopic observation was carried out by directly observing the physical condition of curry leaves (*Murraya koenigii* L.). The results showed that curry leaves have long stems with an odd number of leaflets, ranging from 21 to 22 per stem. The leaves are 2–4 cm long and 1–2 cm wide, oval-shaped with pointed tips, dark green in color, have a distinctive odor, and have a distinctive leaf surface.

3.3. Microscopic

Microscopic examination shows the presence of specific fragments in the form of lithocyst cells containing triangular prism-shaped calcium oxalate crystals. The lithocytic cells resemble a honeycomb with cellulose walls and calcium oxalate crystal deposits [19]. In addition, elongated glandular trichomes, sclerenchyma tissue as reinforcement, and essential oil glands that function as part of the secretory glands were found.

3.4. Moisture Content Determination Results

Moisture content determination is one of the important parameters in simplisia characterization. The results of moisture content testing of curry leaf simplisia powder using the azeotropic method obtained a result of 5%. This value is consistent with the literature, which states that the moisture content of curry leaf crude drug ranges from 8% [20]. Drying in the crude drug manufacturing process aims to reduce the moisture content to maintain quality. High moisture content can cause the crude drug to be easily contaminated by microbes and suffer physical damage [21].

3.5. Curry Leaf Ethanol Extract Results

From 1000 grams of masered curry leaf simplisia powder, 50 grams of thick ethanol extract was obtained with a yield of 7.14%. The extract produced was blackish green in color.

3.6. Phytochemical Screening Results

Phytochemical screening was conducted to identify the groups of secondary metabolites present in the crude drug and its extract. The compounds tested included alkaloids, flavonoids, saponins, tannins, triterpenoids/steroids, and glycosides. The results of the examination are shown in Table 1 below.

Based on Table 1, it is known that both the curry leaf crude drug and its ethanol extract contain several types of secondary metabolites, namely alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides. The presence of alkaloids is indicated by the formation of a white precipitate after the addition of Mayer's reagent, a blackish precipitate with Bouchardat's reagent, and a blackish-brown precipitate after the addition of Dragendorff's reagent. Flavonoid content is detected by the appearance of an orange color in the amyl alcohol layer.

Table 2. Results of phytochemical screening of curry leaf simplisia and ethanol extract.

No	Compound Classes	Curry Leaf Simplisia	Curry Leaf Ethanol Extract
1	Alkaloid	Positive	Positive
2	Flavonoid	Positive	Positive
3	Saponin	Positive	Positive
4	Tanin	Positive	Positive
5	Triterpenoid/Steroid	Positive	Positive
6	Glikosida	Positive	Positive

The presence of steroids and triterpenoids in the curry leaf simplisia and ethanol extract was tested using the Liebermann–Burchard method, a colorimetric qualitative reaction. In this method, the sample is mixed with acetic anhydride and concentrated sulfuric acid. A blue-green coloration indicates steroids, whereas a red or purple coloration indicates triterpenoids. The results showed that both the simplisia and the ethanol extract produced a positive reaction, confirming the presence of steroid or triterpenoid compounds.

The results of the saponin test indicate that both the curry leaf crude drug and the ethanol extract contain saponins. Tannins are identified by a blackish-green color change after the addition of Iron (III) chloride. Steroids/triterpenoids are indicated by the formation of a red or blue-green color, while a positive glycoside compound is indicated by the formation of a brick-red precipitate after heating with Fehling's A and Fehling's B solutions.

3.7. *Results of evaluation of ointment preparations*

Evaluation of the ointment formulated from curry leaf extract included several tests, including organoleptic testing, homogeneity, stability, pH, adhesion, spreadability, potential irritation to the skin of volunteers, hedonic testing, and the ointment's effectiveness in accelerating wound healing in male white mice.

3.8. *Organoleptic Test Results of Curry Leaf Ethanol Extract Ointment (EEDC)*

Organoleptic test observations of ointment preparations containing curry leaves as a coloring agent included color, aroma, and texture. The organoleptic test results are shown in Table 3 below:

Table 3. Organoleptic Test Results of Curry Leaf Ethanol Extract Ointment (EEDC)

Formulation	Color	Aroma	Texture
Blanko	White	Unscented	Semi solid
Ointment EEDC 5%	Dark green	Curry Leaf Specialty	Semi solid
Ointment EEDC 10%	Moss Green	Curry Leaf Specialty	Semi solid
Ointment EEDC 15%	Brown	Curry Leaf Specialty	Semi solid

Information:
Blank: Without curry leaf ethanol extract.
EEDC: Curry leaf ethanol extract

Based on observations, the curry leaf ethanol extract (EEDC) ointment has a semi-solid texture with a distinctive curry leaf aroma. The color of the ointment varies depending on the extract concentration used: white in the blank (without extract), Dark green at a 5% concentration, Moss Green at a 10% concentration, and Brown at a 15% concentration.

3.9. *Homogeneity Testing*

The homogeneity testing results showed that the EEDC ointment at concentrations of 5%, 10%, and 15% did not exhibit any coarse particles or lumps. This indicates that the formulated ointment has a homogeneous composition.

3.10. Stability Testing

Stability evaluation was conducted for 8 weeks by observing changes in color, odor, and texture. The results showed that the EEDC ointment at concentrations of 5%, 10%, and 15% maintained its light brown, brown, and dark brown colors, respectively, while the blank remained white. The distinctive curry leaf aroma was also detected in the EEDC preparation until week 8, while the blank had no aroma. In terms of form, all preparations, both blank and those containing extracts, remained in semi-solid form throughout the test period.

3.11. Wound Healing Test Results

To support the goal of treating superficial wounds, an oily ointment base was used. This base was chosen based on its water-resistant properties, long-lasting durability, and stability and neutrality. Furthermore, oily ointment bases are classified as epidermal ointments with minimal penetration into the skin, thus focusing the therapeutic effect on the injured area [22]. The average results of the curry leaf ethanol extract (ECE) ointment effectiveness test on wound healing are presented in Table 4.

Table 4. Average percentage of incision Wound Healing

Formulation	**Average Wound Healing**			
	Day-2	**Day-5**	**Day-8**	**Day-14**
Blanko	6.00	11.2	33.2	42.62
Ointment EEDC 5%	7.08	16.8	47.7	68.4
Ointment EEDC 10%	9.07	30	67.7	77.30
Ointment EEDC 15%	9.22	56.4	70.9	100
Betadine 10%	8.4	25.8	44.8	80

Description
Blank: Without curry leaf ethanol extract
EEDC: Curry leaf ethanol extract
Comparison: Betadine 10%

3.12. Wound Healing Test Results

Based on average observation results, the blank group demonstrated a relatively slow wound healing process. The percentage of wound healing on day 2 was 6%, increasing to 11.2% on day 5, 33.2% on day 8, and 42.62% on day 14, but complete healing had not yet been achieved. This confirms that the ointment formula without curry leaf ethanol extract has low healing effectiveness.

Conversely, the curry leaf ethanol extract ointment (EEDC) group demonstrated better wound healing effectiveness with varying concentrations. At a concentration of 5%, wound healing reached 7.08% on day 2, increasing to 16.8% on day 5, 47.7% on day 8, and 68.4% on day 14. The 10% concentration gave higher results, namely 9.07% on the day 2, 30% on the 5th day, 67.7% on the 8th day, and 77.30% on the 14th day.

Meanwhile, at the highest concentration (15%), wound healing effectiveness increased significantly, reaching 9.22% on day 2, 56.4% on day 5, 70.9% on day 8, and reaching 100% on day 14, confirming complete wound healing. For comparison, Betadine 10% ointment only showed a healing rate of 80% on day 14.

Thus, the results of this study indicate that the 15% concentration of EEDC ointment is most effective in accelerating wound healing, even surpassing the effectiveness of the comparison ointment (Betadine 10%).

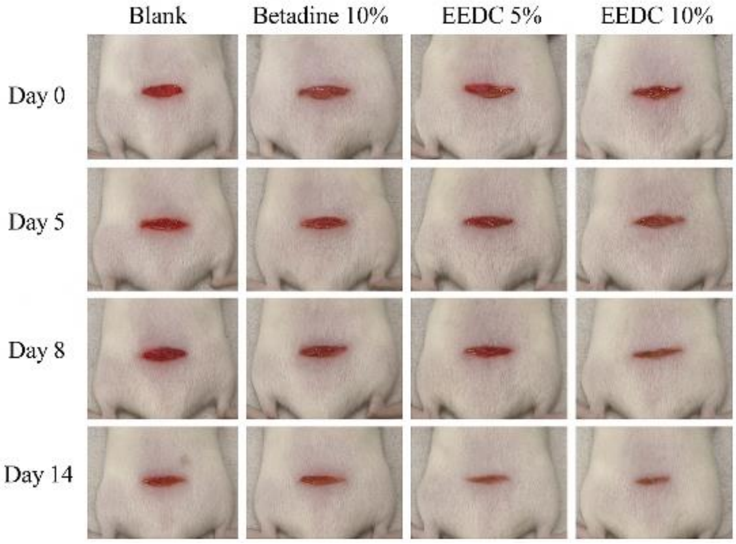

Figure 1. Incision wound healing graph. Incision wound healing graph

3.13. Analysis of Wound Healing Test Results

Figure 1 shows that the percentage of wound healing in white mice increased across all treatment groups. The group with the 15% EEDC ointment concentration showed the most optimal results, with healing reaching 100% by day 12. In contrast, the blank group, as well as the groups with 5% and 10% EEDC ointment concentrations, had not shown complete healing by day 14. This indicates that the 15% EEDC ointment was more effective than the 5% and 10% concentrations.

The statistical analysis using a one-way ANOVA test showed a significance value of 0.00 ($p < 0.05$), indicating a significant difference between the treatment groups. Significant differences were found in the 15% EEDC ointment group compared to the comparison group (10% Betadine), as well as the 10% and 5% EEDC ointment groups. Because the ANOVA results showed significant differences, the analysis continued with a Least Significant Difference (LSD) test to determine which groups were significantly different.

Based on the research results, it can be concluded that the ethanol extract of curry leaves contains chemical compounds that play a role in accelerating the healing of cuts, as indicated by the reduction in wound length in male white mice [23]. Previous research reported that *C. cujete* leaves have anti-inflammatory activity [24] and are able to accelerate the cessation of bleeding in wounds [25]. In addition, binahong leaf extract (*Anredera cordifolia*) in the form of ointment preparations with varying concentrations of 5%, 10%, and 15% using a vaseline base and adeps lanae showed that the effective concentration was at 15%. In contrast to the aforementioned studies, this study used curry leaves (*Murraya koenigii* L.) as the main ingredient in the wound healing test. The superior healing effect observed at the 15% concentration is closely related to the phytochemical constituents detected in the extract. Flavonoids and phenolic tannins function as potent antioxidants that reduce oxidative stress by scavenging reactive oxygen species (ROS), which prevents cellular damage and shortens the inflammatory phase, [26], [27]. Alkaloids, flavonoids, and triterpenoids contribute to anti-inflammatory activity by inhibiting pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and suppressing NF-κB signaling pathways, thus promoting a faster transition to the proliferative phase, [28], [29]. Saponins and tannins exhibit antimicrobial effects that help prevent wound-site infection, thereby enabling uninterrupted tissue repair, [30]. Moreover, flavonoids, triterpenoids, and glycosides stimulate fibroblast proliferation, collagen synthesis, angiogenesis, and keratinocyte migration—key biological processes involved in granulation tissue formation and re-epithelialization, [31], [32]. Steroids and triterpenoids also regulate extracellular matrix remodelling by modulating matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs), supporting proper tissue maturation, [33]. These combined biochemical actions explain the superior wound-healing effect of the 15% curry leaf ethanol extract formulation compared with lower concentrations and the positive control.

4. CONCLUSION

Based on the research results, it can be concluded that both the crude drug and the ethanol extract of curry leaves (*Murraya koenigii* L.) contain secondary metabolite compounds in the form of alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides. The effectiveness test showed that the 15% concentration of curry leaf ethanol extract ointment was the most optimal in accelerating the healing of cuts in male white rats (*Rattus norvegicus*), with a healing rate reaching 100% on the 12th day. Comparative analysis between groups showed a significant difference between the 15% EEDC ointment and the 10% Betadine ointment, with a difference in effectiveness of 20%. In addition, the 10% and 5% EEDC ointment groups also showed significant differences compared to the 10% Betadine ointment, with differences in effectiveness of 2.7% and 11%, respectively. This finding confirms that the 15% EEDC concentration has superior potential compared to lower concentrations and comparison preparations.

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