

Wound Healing Effect of Methanol Extract of Knop Grass (*Hyptis capitata* Jacq.) on Male Mice

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ABSTRACT

Wound healing is a complex physiological process that can be delayed by infection and inflammation. *Hyptis capitata* Jacq., known locally as Knop grass, contains phytochemicals such as flavonoids, tannins, alkaloids, saponins, and steroids, which possess antibacterial and anti-inflammatory properties. This study aimed to evaluate the effect of methanol extract of *H. capitata* leaves on the healing of incisional wounds in male mice (*Mus musculus*). Twenty mice were divided into four groups: a positive control (10% povidone iodine) and three treatment groups receiving 10%, 20%, and 30% methanol extract topically. Wounds were observed daily, and healing duration was recorded. Phytochemical screening confirmed the presence of active secondary metabolites in the extract. The results showed a concentration-dependent effect on healing time, with the 30% extract group healing significantly faster (9.60 ± 0.55 days) compared to the control (14.00 ± 0.00 days). One-way ANOVA and LSD post hoc analysis confirmed significant differences between all groups ($p < 0.001$). In conclusion, the methanol extract of *H. capitata* leaves effectively accelerated wound healing in mice, with the 30% concentration being the most effective. Further studies involving histological evaluation and formulation development are recommended to support its potential as a standardized topical wound-healing agent.

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Keywords:

Hyptis capitata; Wound healing; Incision wound; Methanol extract; Mice; Phytochemicals

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1. Introduction

The skin is the outermost and largest organ of the human body, serving as a protective barrier against harmful external agents, a sensory organ, and a regulator of body temperature [1]. With aging, the skin becomes thinner, making it more susceptible to damage and infection. One common disruption of skin integrity is wound formation.

Wounds occur due to damage to the tissue or anatomical structure of the skin, caused by physical trauma from sharp or blunt objects, medical procedures, animal bites, burns, chemical exposure, or physiological changes [2]. While wounds generally heal naturally, certain factors can hinder the healing process, such as infection, hematomas, and the presence of foreign bodies [3]. Wounds are broadly classified as open or closed. Closed wounds involve internal tissue injury without skin rupture, such as contusions or dislocations, while open wounds involve skin rupture, including abrasions, lacerations, gunshot wounds, and incisions.

Among various wound types, incision wounds (*vulnus scissum*) are common and typically result from surgical procedures involving sharp instruments. These wounds are characterized by straight and well-defined edges [4]. Inflammatory responses often

follow, necessitating topical medications with anti-inflammatory properties. If not treated appropriately, infection may occur and delay healing. The standard topical antiseptic used in first aid is povidone iodine, valued for its antimicrobial properties. However, povidone iodine may cause local side effects such as irritation, redness, or itching in hypersensitive individuals [5].

To improve the safety and efficacy of wound treatment, there is growing interest in exploring herbal remedies with traditional usage and bioactive efficacy. *Hyptis capitata* Jacq., commonly known as Knop grass and referred to as Dungo Herani in Gorontalo, is one such plant. It has long been used by indigenous communities in Indonesia to manage various ailments including fever, headaches, gastrointestinal disorders, diabetes, and external wounds [6]. Its leaves, stems, young shoots, and flowers are processed by boiling, steeping, crushing, or direct application on open wounds.

Phytochemical investigations of the methanol extract of Knop grass have revealed the presence of alkaloids, tannins, and flavonoids—compounds known to possess antioxidant, anti-inflammatory, and antimicrobial activity. Additionally, saponins are reported to stimulate collagen synthesis, an essential component in tissue regeneration [7]. Other studies also identify steroids in the leaves, which may contribute to anti-inflammatory effects by reducing local swelling and inflammatory cell infiltration.

Based on this background, the present study was conducted to evaluate the wound healing effects of methanol extract from *H. capitata* leaves on incision wounds in male mice and to determine the most effective concentration for accelerating the healing process.

2. Methods

Study Design

This study was a laboratory-based experimental design aiming to evaluate the wound healing effect of methanol extract of Knop grass (*H. capitata*) on incision wounds in male mice (*Mus musculus*). A total of 20 healthy male mice weighing 25–30 g were used and acclimatized for 7 days prior to treatment. The animals were randomly divided into four groups (n = 5 per group):

Group I (Positive Control): treated with 10% povidone iodine,

Group II: treated with 10% methanol extract of *H. capitata* leaves,

Group III: treated with 20% methanol extract,

Group IV: treated with 30% methanol extract.

Materials

The materials used in this study included distilled water (aquadest), methanol, acetic acid, concentrated HCl, magnesium powder, Dragendorff's reagent, FeCl₃, Liebermann-Burchard reagent, lidocaine injection, 10% povidone iodine, Knop grass leaves (*H. capitata*), hair removal cream, ethanol, and hot water.

Preparation of Knop Grass Extract

A total of 500 grams of dried Knop grass leaf powder was immersed in 1,700 mL of methanol and extracted using maceration for 3 × 24 hours with periodic stirring. After filtration, the filtrate was concentrated using a rotary evaporator at 40°C to obtain a thick extract. The yield was calculated using the following formula [10]:

$$(\% \text{Yield}) = \frac{\text{Weight of extract obtained}}{\text{Initial simplisia weight}} \times 100\%$$

Phytochemical Screening

Phytochemical screening of the methanol extract of *H. capitata* leaves was conducted using standard methods:

Alkaloids: 2 mL of extract were evaporated, and the residue dissolved in 5 mL of 2 M HCl. The solution was divided into three tubes: one blank, one with Dragendorff's reagent (orange precipitate), and one with Mayer's reagent (yellow precipitate) [8].

Flavonoids: 2 mL of extract were boiled with hot water and filtered. The filtrate (5 mL) was mixed with magnesium powder and 1 mL concentrated HCl. A red, yellow, or orange color indicated a positive result [8].

Tannins: 1 mL of extract was treated with 10% FeCl₃. A greenish-black or dark blue color indicated the presence of tannins [8].

Steroids dan Triterpenoids: 2 mL of extract were mixed with glacial acetic acid and concentrated H₂SO₄. A blue-green color indicated steroids; a red or purple color indicated triterpenoids [8].

Saponins: 2-3 mL of extract were mixed with 10 mL hot water, cooled, shaken vigorously, and treated with 1 drop of 2 N HCl. Stable foam (1-10 cm) lasting ≥10 minutes indicated saponins [8].

Incision Wound Healing Test

Each mouse had its dorsal fur shaved (approx. 4 cm), disinfected with 70% ethanol, and anesthetized via subcutaneous injection of lidocaine. After 3 minutes, a 1.5 cm full-thickness incision was made on the dorsal midline, reaching the subcutaneous layer [9].

Treatment Administration

Topical treatments (1-2 drops) were applied once daily according to group assignment. The healing process was observed daily until full wound closure, defined as complete epithelialization without scab formation.

Data Analysis

The duration of wound healing (in days) was recorded for each group and analyzed using one-way ANOVA to evaluate the effect of treatment. Post hoc analysis using the Least Significant Difference (LSD) test was performed to assess pairwise differences between groups. Normality and homogeneity of variance were tested prior to ANOVA to ensure the validity of the statistical assumptions. A *p*-value < 0.05 was considered statistically significant.

Ethical Consideration

This study was approved by the Health Research Ethics Commission of Universitas Negeri Gorontalo under approval number 221/UN47.7.7/KE/2024, dated 18 October 2024, and conducted in accordance with the 3Rs principles for animal research.

3. Result and Discussions

Extraction Yield of Knop Grass

The maceration method was chosen for its simplicity, low cost, and suitability for thermolabile compounds, as it operates at room temperature without requiring special equipment [10]. Methanol was selected as the solvent due to its ability to penetrate plant tissues and extract a wide range of polar and nonpolar compounds, and its volatility facilitates easy removal during evaporation [11]. The extraction of 500 grams of dried *H. capitata* leaves yielded 65.8 grams of thick extract, corresponding to a percentage yield

of 13.16%. This is considered a good yield, as values above 10% are typically regarded as efficient for plant extractions [12]. This procedure aligns with standardized phytochemical protocols, where solvent polarity and extraction technique play crucial roles in isolating bioactive components [13].

Phytochemical Screening

Phytochemical screening of the methanol extract of *H. capitata* leaves revealed the presence of flavonoids, alkaloids, tannins, saponins, and steroids, while terpenoids were not detected (**Table 1**). The presence of these secondary metabolites was determined through standard reagent-based assays observing specific color reactions [8]. Flavonoids were identified via magnesium and HCl tests, consistent with previous studies showing yellow to green color changes due to complex formation between polyhydroxyl groups and magnesium ions [14]. Alkaloids reacted positively with Dragendorff's reagent, producing an orange precipitate as a result of interaction with tetraiodobismuthate ions [15].

Table 1. Results of Phytochemical Screening of *H. capitata* Methanol Extract

Phytochemical Test	Reagent(s)	Positive Indicator	Result on <i>H. capitata</i>
Flavonoids	Magnesium and HCl	Solution turns yellowish green	Positive
Alkaloids	Dragendorff's reagent	Formation of red-orange precipitate	Positive
Saponins	Warm water and HCl	Formation of persistent foam/froth	Positive
Tannins	FeCl ₃	Solution turns greenish-black or dark blue	Positive
Terpenoids	Liebermann-Burchard reagent	No red ring formation	Negative
Steroids	Liebermann-Burchard reagent	Formation of bluish-green ring	Positive

The formation of greenish-black coloration with FeCl₃ confirmed the presence of tannins, attributed to complexation with Fe³⁺ ions [16]. Saponins were identified through the formation of persistent foam, resulting from the interaction of hydroxyl groups with water molecules [17]. This reaction is further stabilized by interactions with air, producing a lasting froth upon shaking [18]. A bluish-green color observed with Liebermann-Burchard reagent indicated the presence of steroids [19], whereas the absence of a red ring suggested that triterpenoids were not present [20].

Wound Healing Duration

The average wound healing time in the control group treated with 10% povidone iodine was 14.0 ± 0.00 days, while Groups II, III, and IV, which received 10%, 20%, and 30% methanol extract of *H. capitata* leaves, showed healing durations of 13.6 ± 0.55, 11.6 ± 0.55, and 9.6 ± 0.55 days, respectively (**Table 2**). Although povidone iodine is a widely used antiseptic with broad-spectrum antimicrobial activity [21], it may reduce the tensile strength of skin tissue and delay re-epithelialization [22], and it can also cause local irritation due to its foreign molecular structure [23].

These results suggest a dose-dependent wound healing effect. The extract-treated groups exhibited significantly faster healing times compared to the control, and the differences were statistically significant based on one-way ANOVA ($p < 0.05$). This enhancement is likely due to the presence of secondary metabolites in *H. capitata* such as flavonoids, tannins, steroids, and saponins [24]. These compounds possess antibacterial anti-inflammatory, antioxidant [24], and wound-regenerative properties that support all stages of the healing process [25].

Table 2. Wound Healing Duration (Mean \pm SD) in Mice

Group	Treatment	Mean \pm SD (days)
Group I (Control)	10% Povidone iodine	14.00 \pm 0.00 ^a
Group II	10% <i>H. capitata</i> extract	13.60 \pm 0.55 ^b
Group III	20% <i>H. capitata</i> extract	11.60 \pm 0.55 ^c
Group IV	30% <i>H. capitata</i> extract	9.60 \pm 0.55 ^d

Note: Values with different superscript letters differ significantly ($p < 0.05$) based on one-way ANOVA followed by LSD post hoc test

Flavonoids contribute to hemostasis by promoting vasoconstriction and blood clotting. In the inflammatory phase, they inhibit NF κ B expression, reduce inflammatory mediators, and disrupt bacterial membranes. During the proliferative phase, they enhance re-epithelialization and oxygen delivery to the wound site [26]. Tannins further support healing by increasing fibrinogen, stimulating platelet aggregation, and promoting capillary and fibroblast growth, while their astringent properties damage bacterial cell walls during infection [26]. Alkaloids exhibit antibacterial activity by disrupting the peptidoglycan layer of bacterial cells, particularly during the inflammatory stage [26]. Additionally, saponins are known to stimulate collagen formation, essential for tissue regeneration [25].

Therefore, the significantly faster healing observed in the 30% extract group is likely due to the synergistic actions of these compounds, which enhance each phase of the wound healing process.

Results of Research Data Analysis

The wound healing duration data were analyzed using one-way analysis of variance (ANOVA) to determine whether significant differences existed between treatment groups [5]. Prior to the ANOVA test, assumptions of normality and homogeneity were assessed. The data were normally distributed ($\text{sig} > 0.05$), and 9 of the 14 observation days showed homogeneity of variance, supporting the use of ANOVA. For the remaining 4 days, which did not meet the homogeneity assumption ($\text{sig} < 0.05$), the Games-Howell post hoc test was also employed.

Table 3. One-Way ANOVA Summary for Wound Healing Duration

Source of Variation	Sum of Squares (SS)	df	Mean Square (MS)	F-value	p-value
Between Groups	70.53	3	23.51	78.38	< 0.001
Within Groups	4.80	16	0.30		
Total	75.33	19			

Note: $p < 0.001$ indicates a highly significant difference in wound healing duration between groups.

One-way ANOVA confirmed significant differences between the groups ($p < 0.001$; **Table 3**). LSD post hoc analysis showed that each extract concentration significantly outperformed the control. These findings support a dose-dependent effect of *H. capitata* extract on wound healing.

The enhanced healing response may be attributed to the bioactive compounds present in *Hyptis capitata*, including flavonoids, alkaloids, saponins, and phenols [24]. These secondary metabolites are known to play important roles in wound healing by exerting antimicrobial, antioxidant, and anti-inflammatory effects [25]. Flavonoids, for instance, reduce inflammatory mediators and reactive oxygen species, promote vasoconstriction and clot formation during the hemostatic phase, and accelerate re-epithelialization during the proliferative phase [27]. Tannins and alkaloids contribute by enhancing fibrinogen levels, stimulating fibroblast activity, and damaging bacterial cell walls, thereby supporting tissue regeneration [28].

The statistical significance of the differences observed confirms the effectiveness of *H. capitata* extract as a natural wound healing agent. At a 95% confidence level ($\alpha = 0.05$), the null hypothesis was rejected. As stated by Kulsum (2020), ANOVA is a robust method for evaluating differences among group means with minimal error risk, especially when followed by appropriate post hoc testing [29]. Data analyzed using this method must first meet the requirements of normal distribution and homogeneity. Since both conditions for the One-Way ANOVA test have been met, the analysis proceeded with the One-Way ANOVA test.

Although the results were statistically significant, this study has several limitations. First, the sample size was relatively small ($n = 5$ per group), which may limit the generalizability of the findings. Second, the assessment of wound healing relied solely on macroscopic observation without histopathological or molecular confirmation. Third, the study did not investigate potential systemic toxicity or side effects of the extract. Future research should involve larger experimental animals, histological and molecular evaluations, as well as comprehensive safety profiling.

4. Conclusions

Based on the results of this study, it can be concluded that the methanol extract of *H. capitata* leaves is effective in accelerating incisional wound healing in male mice (*Mus musculus*), with the 30% concentration showing the most significant effect as evidenced by the shortest healing duration. Further studies are recommended to investigate the histological mechanisms involved, assess the extract's safety profile for long-term use, and explore its potential development into standardized topical formulations for broader clinical application.

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Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this article.

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