

Effectiveness Ethanol Extract of *Gynura segetum* (Lour.) Merr. Leaves on Incision Wound Healing in Male White Mice (*Mus musculus*)

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Abstract. A wound is damage to tissue continuity caused by injury or surgery. Wounds can be classified into acute and chronic wounds, one type of acute wound being an incision wound, which results from a cut made by a sharp object. One medicinal plant that can be used for treatment is daun dewa (*Gynura segetum* [Lour.] Merr.), which has properties that are highly beneficial and supportive in the care of clean wounds. It contains flavonoid compounds that act as antimicrobial agents, saponins that act as anti-inflammatory, antibacterial, and antifungal agents useful in the wound-healing process, and essential oil that acts as an antibacterial agent. This study aimed to determine the effectiveness of 96% ethanol extract of daun dewa on the healing of incision wounds in male white mice (*Mus musculus*), using an experimental pretest-posttest design. Extraction was performed by maceration with 96% ethanol. Male white mice were randomly divided into five groups: a negative control, a positive control, and treatment groups 1, 2, and 3. The mice were anesthetized with 0.1 mL of ketamine intramuscularly; an incision 2 cm long and 2 mm deep was then made on the shaved and cleaned back of each mouse. The wounds received a positive control of povidone iodine, or extract concentrations of 25%, 50%, and 75%, and were observed for 14 days. Phytochemical screening of daun dewa showed positive results for flavonoids, saponins, and essential oil. On day 14, healing percentages were 93% for K(+), 92% for P3, 83% for P2, and 71% for both P1 and K(-). The effective concentration of 96% ethanol extract of daun dewa for healing incision wounds in male white mice was found to be 50%.

Keywords: *Gynura segetum* (Lour.) Merr.; incision wound; wound healing

INTRODUCTION

A wound is damage to tissue continuity caused by injury or surgery. Wounds can be classified according to anatomy, type, healing process, and healing time. Types of wounds include incision wounds, bruises, abrasions, puncture wounds, scratches, penetrating wounds, and burns (Aminuddin *et al.*, 2020). Wounds can also be classified into acute and chronic wounds, and one type of acute wound is an incision wound, caused by a cut from a sharp object.

Wound healing is a complex process involving continuous biocellular and biochemical activity (Purnama *et al.*, 2017). Natural wound healing occurs through the inflammatory, proliferative, and maturation phases. These three phases influence one another, and many cells and cytokines play a role in each phase (Primadina *et al.*, 2019).

Wounds can be treated using chemical treatments or traditional medicine. For example, povidone iodine is a topical antibacterial compound that effectively kills bacteria and spores and is widely used as a skin disinfectant (Sembiring, 2019). Povidone iodine not only has a broad-spectrum antibacterial effect but also neutralizes the inflammation caused by both pathogens and the host response.

One medicinal plant that can be used for treatment is daun dewa (*Gynura segetum* [Lour.] Merr.), which has antibiotic, anti-inflammatory, antibacterial, and antioxidant properties due to its chemical content, including increased levels of flavonoids, saponins, and essential oil, which play an important role in the wound-healing process (Setyoadi & Sartika, 2010). In wound healing, daun dewa contains the active compound saponin, which functions as an anti-inflammatory and antibacterial agent and can be used in the wound-healing process (Novitasari & Putri, 2016). The active compound essential oil acts as an antibacterial agent by disrupting the formation of the cell membrane or wall (Silawati, 2018). The active compound flavonoid works as part of the defense system in the response to infection by microorganisms, so it is not surprising that this compound is effective as an antimicrobial agent against a number of microorganisms (Parubak, 2013).

Based on the description above, the authors were interested in conducting a study on the effectiveness of 96% ethanol extract of daun dewa (*Gynura segetum* [Lour.] Merr.) on incision wounds in male white mice (*Mus musculus*).

METHODS

Equipment. Mouse cages, mouse drinking bottles, razors, scalpels, dropper pipettes, sterile gauze, analytical scales, rulers, chemical glassware (pyrex), beaker glasses (pyrex), trays, a no. 40 mesh sieve, a simplicia blender (Maspion), a drying cabinet, labels, tweezers, spray bottles, stirring rods, jars, filter paper (Whatman), cotton (Selection), a stopwatch, a macerator, a rotary evaporator, a moisture balance, a waterbath, and cotton buds (Cusson).

Materials. Daun dewa (*Gynura segatum* [Lour.] Merr.) leaves obtained from Pati Regency, Central Java; Betadine ointment (Mundipharma); Vaseline (Unilever); distilled water; 96% ethanol; 0.9% NaCl; ketamine injection (OGB Dextra); Mg powder; concentrated HCl (Xilong Scientific); HCl; and Sudan III.

Moisture Content Test of Simplicia Powder. Moisture content of daun dewa powder was determined by weighing 1 gram of powder into an aluminum sample pan and measuring it with a moisture balance, replicated three times.

Phytochemical Screening. Flavonoid test: 0.5 g of extract was mixed with 10 mL of 96% ethanol, then 0.1 g of Mg powder and 2 drops of concentrated HCl were added; a red or orange color indicates flavonoids. Saponin test: 0.5 g of extract was mixed with 10 mL of hot distilled water and shaken until foaming, then 3 drops of HCl were added and shaken again; stable foam indicates saponins. Essential oil test: 0.5 g of extract was mixed with 10 mL of 96% ethanol and 3 drops of Sudan III; a characteristic odor indicates the presence of essential oil.

Extract Preparation. A total of 5 kg of fresh daun dewa leaves from Pati, Central Java, were washed under running tap water, drained, and dried in a drying cabinet at 50°C for 24 hours. The dried simplicia was ground and sieved (mesh no. 40); 500 g of the resulting powder was macerated in a dark bottle with 3000 mL of 96% ethanol (ratio 1:6) for 24 hours with occasional stirring, then filtered through flannel cloth. The residue was re-macerated three times with the same solvent until clear. The combined macerate was concentrated on a waterbath at 40°C to obtain a thick extract, which was weighed and its yield calculated using the following formula (Dewatisari *et al.*, 2018):

$$\% \text{ Extract Yield} = (\text{Weight of extract obtained} \div \text{Weight of sample extracted}) \times 100\%$$

Activity Testing. Thirty mice acclimatized for 7 days had their backs shaved, cleaned with 96% alcohol, and were anesthetized with 0.1 mL of ketamine (intramuscular). An incision 2 cm long and 2 mm deep was made on the back of each mouse using a scalpel. Mice were divided into 5 groups of 5: K- (no treatment), K+ (Betadine ointment®), P1 (25% extract), P2 (50% extract), and P3 (75% extract). Wound length was measured with a ruler (cm) on days 1, 7, and 14.

Observation of Wound Healing. Wound length in male white mice (*Mus musculus*) was observed every 7 days over 14 days, measured using a graduated ruler (cm), calculated according to the formula of Pongsuplung *et al.* (2012):

$$\frac{\text{Wound length on Day 0} - \text{Wound length on Day } N}{\text{Wound length on Day 0}} \times 100\%$$

Statistical Analysis. Wound length data were tested for normality (Shapiro-Wilk, $n < 50$, significant if $p > 0.05$) and homogeneity (Levene's test, homogeneous if $p > 0.05$), followed by one-way ANOVA (more than two groups) and Tukey's test to identify significant differences between treatment groups ($p < 0.05$).

Research Flow. (1) Plant determination at the Pharmaceutical Biology Laboratory, Universitas Ahmad Dahlan, Yogyakarta; (2) ethical clearance from Universitas Muhammadiyah Purwokerto, Central Java; (3) sample collection of daun dewa from Blaru Village, Pati District, Pati Regency, Central Java; (4) drying and preparation of simplicia powder; (5) moisture content testing; (6) preparation of 96% ethanol extract; (7) phytochemical screening of the extract; (8) determination of the extract concentrations used; (9) adaptation of test animals; (10) administration of 10% povidone iodine; (11) observation of wound healing; and (12) the euthanasia technique.

RESULTS AND DISCUSSION

Moisture Content Test of Simplicia Powder. The moisture content of the powder was tested using a moisture balance, with 1 gram of powder replicated three times. Results are shown in Table 1. The moisture content obtained met the requirement set by the Indonesian Pharmacopoeia of <10%; excessive moisture content in simplicia powder would make it susceptible to microbial growth (Kemenkes RI, 2017).

Table 1. Moisture Content Test Results of the Simplicia

Replication	Moisture Content (%)	Mean (%) $\pm$ SD
1	2.69	2.43 $\pm$ 0.25
2	2.39	-
3	2.20	-

Extraction of Daun Dewa. A total of 500 g of daun dewa was macerated with 3000 mL of 96% ethanol (ratio 1:6) for 3 $\times$ 24 hours with occasional stirring. Extraction results are shown in Table 2. Maceration was chosen for its simplicity and because it requires no heating, making it suitable for heat-labile compounds (Yuliana, 2016). The 96% ethanol solvent penetrates cell walls more easily than lower-concentration ethanol, producing a more concentrated extract (Wendersteyt et al., 2021). The filtered extract was concentrated on a waterbath at 40°C to prevent compound degradation, yielding 60 g of thick extract (12% yield). A longer extraction time increases yield, since more compounds are extracted through maceration (Wijaya *et al.*, 2018).

Table 2. Extract Yield

Daun Dewa Powder	Extract	Yield
500 g	60 g	12%

Phytochemical Screening. Identification results for compounds in the 96% ethanol extract of daun dewa are shown in Table 3. A positive flavonoid result is indicated by an orange-red color for flavones, dark red for flavanols or flavanones, and green for glycosides (Mariana et al., 2019). A positive saponin result is indicated by stable foam, since saponins are surface-active compounds; the tested sample formed foam 1–10 cm high lasting approximately 10 minutes (Depkes RI, 1995). A positive essential-oil result is indicated by the characteristic odor produced by the residue (Padmasari *et al.*, 2013).

Table 3. Phytochemical Screening Results

Compound Group	Reaction	Phytochemical Result	Color
Flavonoid	Mg + concentrated HCl	+	Orange-red
Saponin	Distilled water + HCl	+	Stable foam formed
Essential oil	Sudan III	+	Characteristic odor

Observation of Incision Wound Length. On day 0, test animals were incised (2 cm long, 2 mm deep) after cleaning with an alcohol swab and anesthesia with 0.1 mL of ketamine. Group K+ received 0.1 mg of povidone iodine daily until day 14, while P1, P2, and P3 received 25%, 50%, and 75% daun dewa extract, respectively. On day 1, no changes were observed in any group; wounds remained wide open with reddish coloration (the inflammatory phase, days 1–4). Table 4 shows the average wound length on day 1.

Table 4. Average Incision Wound Length on Day 1

Day	Group	Average (cm)
Day 1	Negative control	1.88
	Positive control	1.86
	Concentration 25%	1.86
	Concentration 50%	1.84
	Concentration 75%	1.82

On day 7, K(-) wounds remained open and red, as this group received no active treatment, while K(+), P1, P2, and P3 wounds had partially closed, turning brown and dry (the proliferative phase, days 4–7). Table 5 shows the average wound length on day 7.

Table 5. Average Incision Wound Length on Day 7

Day	Group	Average (cm)
Day 7	Negative control	1.16
	Positive control	0.92
	Concentration 25%	1.14
	Concentration 50%	1.1
	Concentration 75%	0.94

On day 14, K(-) wounds remained partly open and reddish, with some drying from natural healing alone. K(+) wounds were dry and fully closed, attributable to the antiseptic action of povidone iodine. In P1, P2, and P3, the wounds showed blackish-to-whitish color changes, nearly closing completely (the maturation phase, days 7–14); in the overall wound-healing process this phase typically spans day 24 to one year and is the longest phase (Aminuddin *et al.*, 2020). Table 6 shows the average wound length on day 14.

Table 6. Average Incision Wound Length on Day 14

Day	Group	Average (cm)
Day 14	Negative control	0.58
	Positive control	0.14
	Concentration 25%	0.58
	Concentration 50%	0.32
	Concentration 75%	0.18

Percentage of Incision Wound Healing. Wound length reduction was measured from an initial 2 cm on day 0 through days 1, 7, and 14; each treatment group showed healing at a different rate. Figure 1 shows the healing-percentage graph, and Table 7 shows the percentage results.

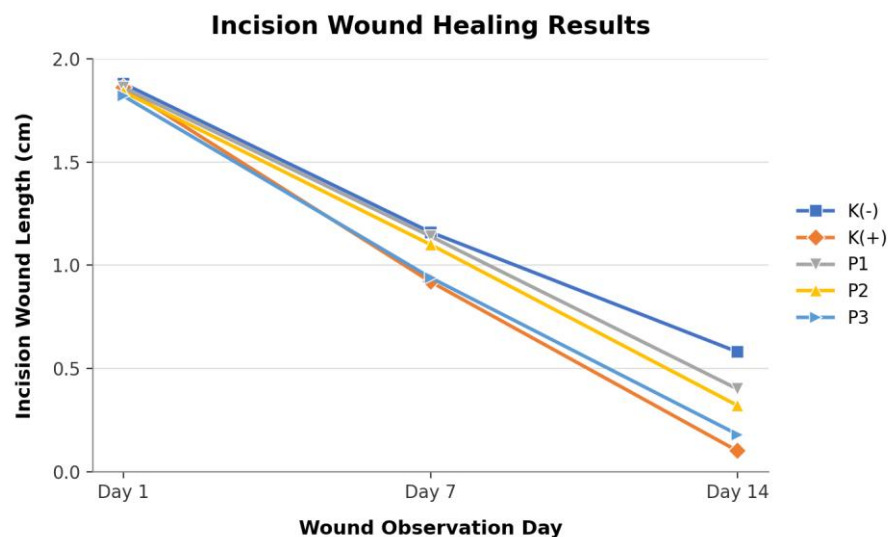


Figure 1. Average Incision Wound Healing Graph

On day 1, the healing percentage had not yet changed in any group (graph at its highest point). By day 7, the graph shows a decrease across groups as healing progressed. By day 14, the graph shows a further decrease, with K(+) and P3 nearly healed, reaching the lowest point of the graph.

Table 7. Incision Wound Healing Percentage Results

Group	Day 1 (%) $\pm$ SD	Day 7 (%) $\pm$ SD	Day 14 (%) $\pm$ SD
K(-)	6 $\pm$ 2.23	42 $\pm$ 5.7 (b)	71 $\pm$ 4.1 (b)
K(+)	7 $\pm$ 2.7	53 $\pm$ 4.4 (a)	93 $\pm$ 8.3 (a)
P1	7 $\pm$ 2.7	43 $\pm$ 5.7 (b)	71 $\pm$ 6.5 (b)
P2	8 $\pm$ 2.7	45 $\pm$ 3.5 (ab)	84 $\pm$ 6.5 (a)
P3	9 $\pm$ 2.2	53 $\pm$ 5.7 (a)	92 $\pm$ 2.7 (a)

Note: (a) = significant difference compared to the negative control ($p < 0.05$); (b) = significant difference compared to the positive control ($p < 0.05$).

Statistical Analysis. Healing percentage data were analyzed with SPSS 22. The Shapiro-Wilk normality test ($n < 50$) showed significance values > 0.05 for all groups, and Levene's test confirmed homogeneity ($p > 0.05$). One-way ANOVA showed no significant difference on day 1 ($p > 0.05$), but significant differences on days 7 and 14 ($p < 0.05$). Tukey's HSD test was then used to compare group means.

On day 1, K(-) did not differ significantly from K(+), and P1, P2, and P3 did not differ from one another, indicating that the extract concentrations had not yet produced a measurable wound-healing effect. On day 7, significant differences ($p < 0.05$) were found between K(-) and K(+), P2, and P3, since K(-) received no treatment while K(+) (povidone iodine) and P2/P3 (50%/75% extract) showed a healing effect; K(+) also differed significantly from K(-), P1, and P2. On day 14, K(-) differed significantly ($p < 0.05$) from K(+), P2, and P3, while K(+) differed significantly from K(-) and P1.

K(-), receiving no active substance, showed no wound-healing effect, and P1 (the lowest concentration) remained equivalent to K(-). K(+) did not differ significantly ($p > 0.05$) from P2 and P3 on day 14, indicating that both concentrations produced effects equivalent to povidone iodine, attributable to their flavonoid and saponin content. Flavonoids halt bleeding through vasoconstriction and act as free-radical scavengers, inhibiting enzymatic hydrolysis and oxidation (Calsum et al., 2018), while saponins reduce inflammation and strengthen skin cells (Setyoadi & Sartika, 2010), and essential oil acts as an antibacterial agent through its hydroxyl functional groups (Silawati, 2018).

CONCLUSION

The 96% ethanol extract of daun dewa (*Gynura segatum* [Lour.] Merr.) is effective in healing incision wounds in male white mice (*Mus musculus*), with an optimal concentration of 50%. Given the limitations of this study, further research is recommended to quantitatively assess the secondary metabolites in daun dewa and to conduct microscopic histopathological observation of skin tissue.

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