

EFFECT OF THE WATER EXTRACT OF THE FOUR O'CLOCK HERB (*MIRABILIS JALAPA* L.) ON THE HEALING OF OPEN WOUNDS IN RATS

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ABSTRACT

Objective: This study aimed to analyze the effect on wound healing following treatment with a water extract of *Mirabilis jalapa* L. by investigating wound contraction and the associated histopathological changes in rat skin.

Methods: Male Sprague-Dawley rats were divided into five groups, namely negative control, positive control (povidone-iodine), dose 1, dose 2, and dose 3. A 20-x10-mm rectangular wound area was created for the test. In dose 1, 2, and 3 groups, the corresponding dose variation of a 0.5-mL *M. jalapa* L. water extract (dose 1: 5% v/v, ≈243.1 mg/kg body weight BW; dose 2: 10% v/v, ≈486.2 mg/kg BW; and dose 3: 20% v/v, ≈972.4 mg/kg BW) was topically applied for 14 days on open wounds of rats. Widespread wound contractions were measured on days 1, 3, 5, 7, 9, 11, and 13, and histopathological changes in the skin were observed on day 15 using hematoxylin and eosin staining.

Results: The *M. jalapa* L. water extract accelerated wound healing. The optimal dose was found to be 20% v/v (≈972.4 mg/kg BW).

Conclusion: *M. jalapa* L. extracts are potential healing agents for open wounds.

Keywords: *Mirabilis jalapa* L., Open wound, Povidone-iodine, Histopathology, Wound healing.

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INTRODUCTION

Wounds are tissue injuries that interfere with normal cellular processes. They involve damage to the unity of the body tissues, usually accompanied by the loss of tissue substance. Open wounds involve damage to the skin or mucous membranes and a possibility of bleeding accompanied by tissue damage and a risk of infection [1]. Wounds are classified as acute or chronic based on their severity, and both types are at the risk of infection. Acute wounds are fast healing, for example, stitched surgical lesions, trauma wounds, and blisters [1]. The most commonly used antiseptic drug for wounds is 10% povidone-iodine, which contains 1% available iodine that can kill bacteria in 1 min and spores within 15 min. However, excessive iodine use may inhibit the wound granulation process. Side effects occur when it is used on a widespread skin surface (e.g., wounds using >0.9 mL/per day) because iodine stimulates and increases serum levels resulting in acidosis, neutropenia, and hypothyroidism [2].

Based on the high prevalence of injuries and side effects caused by existing medicines, the development of an alternative agent for wound healing is necessary. A potential candidate is the four o'clock herb *Mirabilis jalapa* L., an ornamental garden plant. The infusion and decoction of *M. jalapa* L. have been traditionally employed as an anti-inflammatory and pain medication in Brazil. *In vitro* studies have revealed that the flowers primarily contain flavonoids and anthocyanins [3]. In addition, the antibacterial activity of an *M. jalapa* L. extract was investigated using bioautography with *Staphylococcus aureus* and *Escherichia coli* as the test organisms [4-6].

M. jalapa L. contains β -sitosterol-D-glucoside. This natural estrogenic steroid maintains the moisture in the wound area, promoting cell growth. In the inflammatory phase, β -sitosterol limits the amount of prostacyclin, thereby accelerating the inflammatory phase. Flavonoids limit the amount of free radicals, preventing excessive tissue damage in the inflammatory phase [7-9]. The purpose of this study was to analyze

the effects of an *M. jalapa* L. extract on the acceleration of open wound healing. This was assessed based on the percentage of widespread wound contraction and the histopathological changes observed in the skin of rats with open sores to determine the optimal extract dose.

METHODS

Extract preparation

Herbal extracts consisting of flowers, leaves, and tubers of *M. jalapa* L. were supplied by Research Institute for Spices and Medicinal Plants (BALITTRO), Cimanggu, Bogor, West Java. Extraction was performed by water solvent using the maceration method over 7 days. Subsequently, the solvent was removed through evaporation, and a thick extract remained.

Phytochemical screening and determination of total flavonoid content

Phytochemical screening was performed on the extracts to identify the presence of flavonoid, tannin, alkaloid, saponin, phenolic, triterpenoid, steroid, and glycoside compounds. The total flavonoid content was also assessed to determine the flavonoid levels in the extracts that were supposedly responsible for the biological activity to be tested.

Testing materials manufacturing

The herbal extracts of *M. jalapa* L. were sterilized in an autoclave at 121°C for 15 min. Subsequently, they were dissolved in water at room temperature (25°C–27°C) to achieve the required dosage as calculated.

Preparing trial animals

Male Sprague-Dawley male rats were obtained from the Faculty of Agriculture, Bogor Agricultural University, Indonesia. The animals were given food and water *ad libitum* for 14 days to acclimatize to the new environment. During acclimatization, the general state and any weight loss of the rats were observed to select only healthy rats for the study use. This study was approved by the Ethics Committee of Health

Research, Faculty of Medicine, Universitas Indonesia (No. 504/UN2.F1/ETIK/2015).

Determination of extract dose

Preliminary experiments were performed to determine the dose and treatment duration of the *M. jalapa* L. water extract required to affect wound healing. Four rats were used. Two cuts on the upper and lower rumps were made at 3-cm intervals. The eight wounds were randomly divided into four groups. Each group was treated as per Table 1.

Following the injury, the extract was applied daily to the injury site. All wounds were covered with sterile gauze. Daily observations were performed until the wound had healed. In the preliminary trial, the best result was obtained from dose 3, with wound closure on day 14. This optimum dose was used as the middle dose in our study.

Open wound procedure

The rats were shaved in the upper back area before anesthetization using ether. The upper back and surrounding areas were cleaned with 70% alcohol. Subsequently, a rectangular 20×10×2-mm (length×width×depth) wound was made.

Trial implementation

A total of 15 male rats received two wound treatments, each on the upper and lower back separated by 3-cm intervals. Thus, 30 sets of treatment data were obtained from the 15 rats. The 30 wound sites were randomly divided into five treatment groups (Table 2) of six wounds each. A topical dropping technique was used to administer 10 standard drops (0.5 mL) daily at the same time for 14 days.

Wound observation

Widespread vertical and horizontal wound measurements were taken on days 1, 3, 5, 7, 9, 11, and 13. The average area of measurement was used for assessment. The wound was considered rectangular, and the wound area was calculated as follows: Widespread=length×width. The percentage of wound contraction was calculated by dividing (L1 - L2) with L1, then times 100%, where L1 is the initial wound area, and L2 is the wound area on the day of observation.

Histopathological observation

For histopathology, the normal histological features of the skin were compared to determine the extent of wound tissue repair all rats were sacrificed for their skin on day 15 by neck dislocation. The internal organs were removed followed by skin removal. The cut skin sample

was soaked in a 10% formalin buffer solution followed by ethanol dehydration and mounting in a paraffin block. Subsequently, 5- μ m thick samples were cut using a Spencer 1010-SMT-006 microtome (New Delhi, India) and stained with hematoxylin and eosin. Fibroblasts, collagen, angiogenesis, neutrophils, reepithelization, and sebaceous glands were observed under a Leica DM 750 light microscope (Leica Microsystems, Heerbrugg, Switzerland).

Data processing

The percentage of data obtained for wound contraction were tested for normality and homogeneity. Normal and homogeneous data were analyzed using one-way analysis of variance. If the data obtained were not normal or homogeneous, non-parametric analysis was performed by direct data comparison between treatment groups to determine whether the data were significantly different.

RESULTS

Phytochemical screening and determination of total flavonoid content

The *M. jalapa* L. extract screened positive for flavonoids (Table 3). Further testing revealed a 0.87% flavonoid composition.

Test results of wound healing activity

The percentage of wound contraction was calculated on days 1, 3, 5, 7, 9, 11, and 13 (Table 4).

Histopathological analysis

The results of the histopathological analysis of rat skin at day 15 are presented in Table 5 and Fig. 1.

DISCUSSION

Previous research has established that β -sitosterol-D-glucoside is a natural steroid contained in extracts of *M. jalapa* L. [5,6]. However, the results of phytochemical screening in our study were not positive for steroids. This could perhaps be because our extract comprised flowers, leaves, and tubers of *M. jalapa* L., whereas the extracts mentioned in the previous studies could have contained different components or concentrations of *M. jalapa* L. Furthermore, our extract was autoclaved at 121°C before use, and this could have possibly altered the stability of any steroid phytotoxins that were present [10-13].

Based on Table 4, the highest mean wound reduction percentage at day 13 was by dose 3 (97.42%) followed by that by dose 1, the positive

Table 1: Preliminary trial treatment groups

Group	Wound size (2 cm ²)	Treatment
Positive control	Two wounds	Positive control, rats were wounded and given 10% povidone-iodine
Dose 1	Two wounds	Rats wounded and given 0.1% v/v (\approx 4.86 mg/kg BW) <i>M. jalapa</i> L. water extract
Dose 2	Two wounds	Rats were wounded and given 1% v/v (\approx 48.6 mg/kg BW) <i>M. jalapa</i> L. water extract
Dose 3	Two wounds	Rats were wounded and given 10% v/v (\approx 486.0 mg/kg BW) <i>M. jalapa</i> L. water extract

M. jalapa: *Mirabilis jalapa*, BW: Body weight

Table 2: Animal testing groups used

Treatment group	Type of treatment
Negative control	The rats were injured, and the wound was treated with 10 drops (0.5 mL) of solvent daily at the same time for 14 days.
Positive control	The rats were injured, and the wound was treated with drops (0.5 mL) of 10% povidone-iodine solution daily at the same time for 14 days.
Dose 1	The rats were injured, and the wound was treated with 10 drops (0.5 mL) of the <i>M. jalapa</i> L. extract at dose 1 (5% v/v extract, \approx 243.1 mg/kg BW) daily for 14 days.
Dose 2	The rats were injured, and the wound was treated with 10 drops (0.5 mL) of the <i>M. jalapa</i> L. extract at dose 2 (10% v/v, \approx 486.2 mg/kg BW) daily for 14 days.
Dose 3	The rats were injured, and the wound was treated with 10 drops (0.5 mL) of the <i>M. jalapa</i> L. extract at dose 3 (20% v/v, \approx 972.4 mg/kg BW) daily for 14 days.

M. jalapa: *Mirabilis jalapa*, BW: Body weight

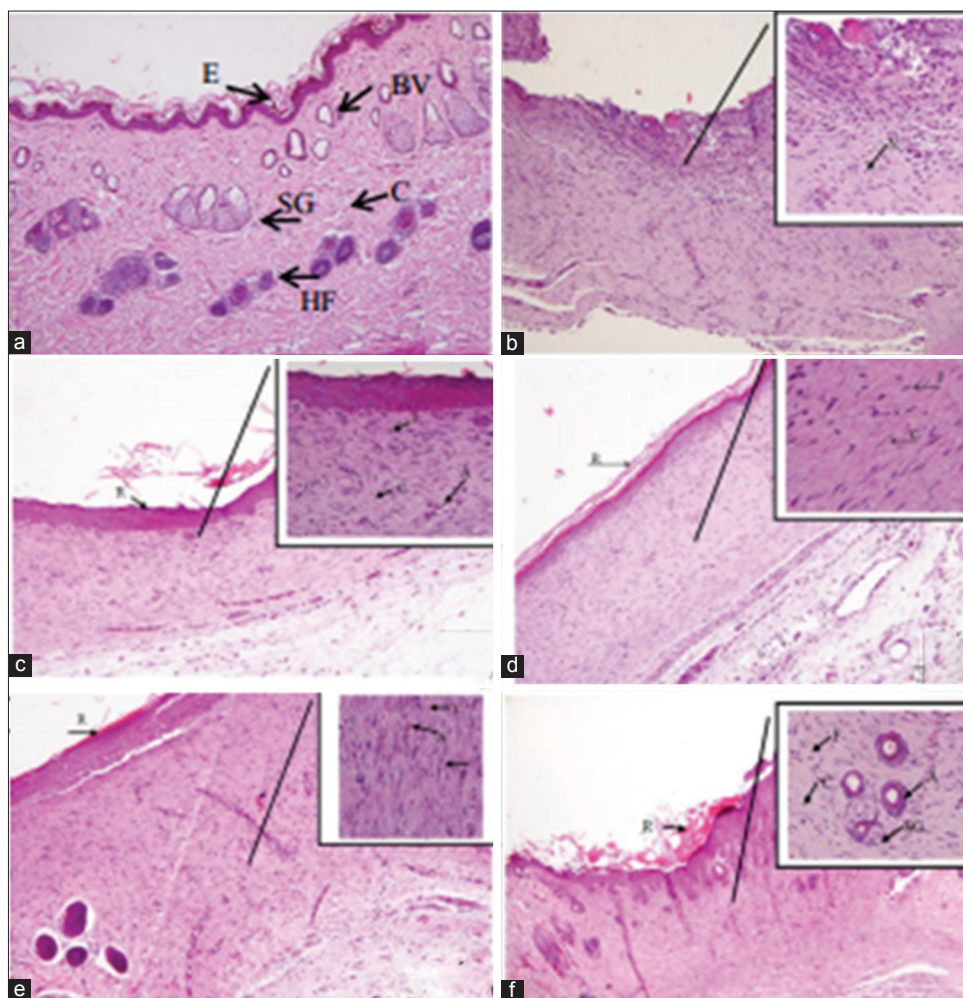


Fig. 1: Histopathologic observations of prepared rat skin stained with hematoxylin and eosin and examined under 100 \times magnification on day 15. (a) Normal control, (b) positive control = 0.5-mL 10% v/v povidone-iodine, (c) negative control = 0.5-mL water, (d) dose 1 = 0.5-mL 5% *Mirabilis jalapa* L. water extract, (e) dose 2 = 0.5-mL 10% v/v *M. jalapa* L. water extract, (f) dose 3 = 0.5-mL 20% v/v *M. jalapa* L. water extract. F = Fibroblasts, C = collagen, R = Reepithelization, N= neutrophil, HF = hair follicle formation, BV= blood vessels, A = angiogenesis, SG = sebaceous glands.

Table 3: Results of four o'clock herbal extract content screening

Class of compounds	Results
Flavonoids	+
Tannins	+
Alkaloids	+
Saponins	+
Phenolics	+
Triterpenoids	-
Steroids	-
Glycosides	+

control, dose 2, and the negative control. This indicates that dose 3 caused the fastest wound contraction from the three *M. jalapa* L. extracts tested.

The statistical test results revealed a significant difference between the results of the negative control and those of the positive control and dose 3 on day 1 ($p < 0.05$), suggesting that a wound-healing effect was already being exerted as early as the 1st day of treatment with dose 3. Meanwhile, the other dose groups demonstrated significantly different results from those of the negative control on day 3.

The open wound healing activity that was observed following treatment with the *M. jalapa* L. extract could have been caused by its high content

of flavonoid compounds. Flavonoids have been known to limit the amount of free radicals that prevent excessive tissue damage in the inflammatory phase [7,9]. Clinically, inflammation is the second phase of the healing process and is characterized by erythema, swelling, and temperature increases that are often associated with pain. Furthermore, the antibacterial activity of the *M. jalapa* L. extract enhances the acceleration of wound healing [14]. This could be particularly useful in treating open wounds, where infection is highly likely and would inhibit the healing process [15].

Based on the histopathological features (Table 5), we concluded that the anti-inflammatory effect of the *M. jalapa* L. water extract was observed at all doses, that is, neutrophils as an inflammatory indicator were not observed. Moreover, indicators of angiogenesis and sebaceous glands were close to normal in the *M. jalapa* L. water extract groups compared with those in the control groups, indicating accelerated wound healing.

A limitation of our study is that only one extract batch was tested. Further studies should investigate multiple extract batches to determine variation in extract composition and establish the optimal composition parameters required for accelerated wound healing.

CONCLUSION

Based on our results, it can be concluded that *M. jalapa* L. extract has the potential to aid wound healing as demonstrated by widespread

Table 4: Calculation results of percentage contraction of the wound area

Treatment group	Average percentage of widespread wound contraction (%)						
	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
Positive control	8.07*	23.14*	38.28*	53.21*	66.92*	82.07*	95.57*
Negative control	1.92	9.42	14.85	26.50	33.50	40.50	48.85
Dose 1 (5% v/v)	4.21	15.21*	36.28*	51.35*	62.07*	82.07*	97.27*
Dose 2 (10% v/v)	8.33*	23.50*	35.83*	56.58*	69.83*	81.50*	95.33*
Dose 3 (20% v/v)	6.07*	20.00*	33.92*	52.57*	66.28*	82.00*	97.42*

The application of the *M. jalapa* L. water extract to open wounds was tested at three different doses. *Significantly different compared to negative control (p<0.05)

Table 5: Comparison of significant histopathological differences between treatment groups on day 15

Group	Data	Indicator						Scoring	Average scoring
		Reepithelization	Fibroblasts	Collagen	Angiogenesis	Sebaceous Glands	Neutrophils		
Normal	1	1	1	1	1	1	0	5	5.0
Positive control	1	1	1	1	1	1	1	6	5.0
	2	1	1	1	1	1	0	5	
	3	1	1	1	1	0	0	4	
Negative control	1	1	1	1	1	0	1	5	3.0
	2	1	0	0	0	0	1	2	
	3	1	0	0	0	0	1	2	
Dose 1	1	1	1	1	0	0	0	3	3.333
	2	1	1	1	0	0	0	3	
	3	1	1	1	1	0	0	4	
Dose 2	1	1	1	1	1	0	0	4	4.333
	2	1	1	1	1	0	0	4	
	3	1	1	1	1	1	0	5	
Dose 3	1	1	1	1	1	1	0	5	5.0
	2	1	1	1	1	1	0	5	
	3	1	1	1	1	1	0	5	

Negative Control=0.5-mL water topically, positive control=0.5-mL 10% v/v povidone-iodine, Dose 1=0.5-mL 5% v/v *M. jalapa* L. water extract, Dose 2=0.5-mL 10% v/v *M. jalapa* L. water extract, Dose 3=0.5-mL 20% v/v *M. jalapa* L. water extract. Scoring: 1=exist qualitatively, 0=none. *M. jalapa*: *Mirabilis jalapa*

contraction parameters and histopathological indicators. An effective dose of 972.4 mg/kg body weight was determined.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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