

# Celery leaf extract cream is a potential wound healing agent

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## Abstract

Natural ingredients have been widely used for wound care and healing because of their flavonoid, saponin, and tannin contents, which can facilitate the healing process. This study aimed to examine the efficacy of celery leaf extract cream in the treatment of cutaneous wounds on the surfaces of white Wistar rats. This study involved laboratory-based experimental research utilizing a pretest-posttest with a control group design. Twenty white rats were sampled for this study and divided into four groups, each consisting of five rats, including one control group and three treatment groups. The extract was obtained via maceration, whereas the celery leaf extract cream contained an oil-in-water emulsion. A one-way Anova test was used to test differences between groups ( $p < 0.05$ ), and a Post Hoc test with LSD technique was conducted to determine the most effective treatment group. Celery leaf extract cream has been found to be more efficient in accelerating skin regeneration and stimulating fibroblast proliferation than base cream. This is evidenced by the superior performance of the celery leaf extract cream treatment group in closing the incision wound, as well as the group's higher production of fibroblasts that are essential for wound healing, compared to the base cream group. This is evidenced by the superior performance of the celery leaf extract cream treatment group in closing the incision wound, as well as the group's higher production of fibroblasts that are essential for wound healing, compared to the base cream group. The anti-inflammatory and antioxidant properties of the flavonoids found in celery leaves can potentially prevent the formation of free radicals and expedite wound healing.

**Keywords:** wound healing, incision wound, celery leaf extract cream

## Introduction

Wounds are a common type of injury that can cause damage to the normal skin epithelial tissue and anatomical structures.<sup>1</sup> There are several types of wounds, such as abrasions, incisions, lacerations or scars, puncture wounds, bites, and burns.<sup>2</sup> An incision is a wound that can occur due to a sharp object. It is characterized by an open wound, pain, and a large, deep, and long incision. An incision can be made by scraping the rough surface. Although not very deep, an incision can injure a wide area of skin to be injured.<sup>3</sup> Open wounds such as incisions are highly susceptible to infection, especially by bacteria, and can serve as entry points for systemic infection. Infected wounds tend to heal more slowly, often leading to the formation of exudates and toxins, along with the death of regenerative cells. Therefore, there is a need to stimulate healing and restore the normal function of the injured body part to prevent infection.<sup>4</sup>

Normally, a wound undergoes a healing process, which is a response of connective tissue. Wound healing is a coordinated process that involves cellular, humoral, and connective tissue elements. In humans, the wound healing process is divided into three overlapping phases: the inflammation phase, new tissue formation phase (proliferative phase), and tissue strengthening phase (maturation or remodeling phase).<sup>3,5,6</sup> Wound healing is important because the skin has specific functions in the body, including protective, sensory, thermoregulatory, metabolic, and sexual signaling. These functions cannot operate as they should when a wound occurs. Wound healing is a biological process that occurs in the body and involves a series of complex, vulnerable, and easily disrupted processes, which may lead to disturbances or

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failures. Therefore, optimal conditions are required for optimal wound healing. The goal of wound management is to minimize tissue damage, provide proper tissue oxygenation and nutrition, reduce risk factors that impede wound healing, accelerate the healing process, and reduce the incidence of infected wounds.<sup>7</sup>

Management of wounds can be achieved by providing antiseptics, antibiotics, and general wound care. Wound management is usually carried out in two ways, namely medically and empirically. Medical treatment is usually performed in areas with adequate health facilities, whereas empirical treatment is often carried out by people living in areas with minimal health facilities. In the case of empirical wound management, plants in the surrounding area are usually used.<sup>8</sup> Wound management and care can also use drugs that are already known among the community, one of which is povidone iodine, an external antiseptic with a microbicidal spectrum for the prevention or treatment of topical infections related to surgery, cuts, abrasions, and reducing mild mucosal irritation.<sup>9</sup> However, povidone iodine can cause contact dermatitis on the skin, has toxicogenic effects on fibroblasts and leukocytes, inhibits neutrophil migration, and reduces monocyte cells.<sup>10-12</sup> Therefore, it is necessary to look for other alternatives for wound healing that are safe, easily obtainable, and effective using natural ingredients.

Natural materials have been widely used for the treatment and healing of wounds owing to the presence of various compounds, such as flavonoids, saponins, and tannins, which play a role in wound healing. These compounds also exhibit antioxidant and pro-angiogenic properties, and can enhance oxygen supply and nourishment to the injured skin.<sup>13</sup> One of the plants that contains these compounds and is utilized for wound healing is celery (*Apium graveolens*). Celery leaves have been traditionally used for various purposes such as rheumatism, dry eyes, hypertension, cough, reducing cholesterol levels, and promoting hair growth<sup>14</sup>, as well as for their antibacterial, antispasmodic, uric acid-reducing, anti-rheumatic, sedative, and antihypertensive properties.<sup>15</sup> Celery contains flavonoids that can act as potential anti-allergic, anti-inflammatory, antiviral, anticarcinogenic, and antioxidant agents.<sup>16</sup> It also contains saponins, tannins, essential oil, apigenin, vitamins A, B, and C<sup>15,17</sup>, iron, calcium, sulfur, and phosphorus<sup>18</sup>. All contents are present in each section of the gel; therefore, each section can be used independently. Djajanti and Asfi<sup>16</sup> concluded that celery leaf extract at concentrations of 2% and 4% has wound healing activity in rabbits. Nikola et al.<sup>19</sup> concluded that the leaf extract of celery leaf (concentrations of 1%, 2%, and 4%) had faster wound healing activity against white muscle tissue granuloma, and the wound healing activity of the 4% cream was comparable to that of the positive control. The background of these studies provides an important reason for researchers to conduct more in-depth experimental laboratory research on the effectiveness of celery leaf extract cream in wound healing on the surface of white muscle tissue (*Rattus norvegicus*) granuloma.

## Method

### Study design

This was a laboratory experimental study using a pretest-posttest with a control group design to analyze the effectiveness of celery leaf extract cream in the wound healing process on the surface of white Wistar rat skin. The study sample consisted of 20 adult male white Wistar rats weighing 160-200 grams and aged 2-3 months. The number of white rats used in this study was considered large enough and divided into four groups, each consisting of five rats, including the control group (P0) and treatment groups 1, 2, and 3 (P1, P2, and P3).

### Materials

The tools used included minor surgical instruments (stainless steel tray, scalpel, blade, scissors, and forceps), a scale, sterile gloves, cotton balls, gauze, a rat cage, a rat feed container, writing tools, markers, and a caliper. The materials used include celery leaf, glycerin, triethanolamine (TEA), ethyl alcohol, acetic acid, methyl paraben, propyl paraben, distilled water, sterile tampons, anesthesia (ketamine), xylazine, Wistar strain white rats, and rat food and drink.

## Procedure

### *Acclimatization of animals*

Before administering the treatment, acclimatization (acclimatization) of all animals was conducted at the Animal House, Faculty of Medicine and Pharmacy, Universitas Sumatera Utara for one week. The animals were acclimatized to new housing and environment, as well as food and water supply according to their needs (libitum).

### *Preparation of celery leaf extract*

Fresh celery leaves were picked, free from dirt, washed with running water until clean, and then dried under a fan heater at 50-60°C. The cleaned and dried leaves were ground into fine powder and mixed with distilled water in a glass jar with a separator. The mixture was shaken until the powder was evenly suspended in the water. The jar was tightly closed and stored for approximately 5 hours, stirred once every hour. The resulting solution was filtered and the process was repeated three times, then stored in a bottle. The extract was concentrated using a rotary evaporator at 70°C to obtain a thick celery leaf extract.

### *Preparation of emulsion cream*

The emulsion cream was prepared using the water-in-oil (W/O) emulsion method. Cream was produced at concentrations of 0% (base), 2.5%, 5%, and 10%. The ingredients were weighed according to Table 1 and mixed with distilled water until the total weight reached 100 g.

Tabel 1. Celery extract cream preparation formula

Materials	Formula/cream dose (gram)			
	F0	F1	F2	F3
Celery extract cream	0	10	15	25
Setil alkohol	4	4	4	4
Gliserin	15	15	15	15
TEA (trietanolamin)	3	3	3	3
Asam stearat	12	12	12	12
Metil paraben	0,2	0,2	0,2	0,2
Propil paraben	0,02	0,02	0,02	0,02
Aquades	100	100	100	100

### *Incision wound making*

The wound area (anterior) was cut to approximately 2 cm<sup>2</sup> (2 cm × 2 cm). After cutting, the cheek was hung with a weight of 80 ml/kg body weight and xylasin 5 ml/kg body weight to prevent pain and excessive movement. The cheek was then cut vertically to a depth of ± 2 mm, until the dermal layer was reached.

### *Wound treatment and observation*

Wound treatment on the skin surface of the rats was provided based on the wound care protocol according to the specified treatment group: a) control group (P0) (the wound was treated with 0% base cream (not containing celery leaf extract) and then covered with gauze (twice a day in the morning and evening); b) Treatment group-1 (P1) (the wound was treated with 2.5% celery leaf extract cream and then covered with gauze (twice a day, morning, and evening); c) Treatment group-2 (P2): the wound was treated with 5% celery leaf extract cream and then covered with gauze (twice a day, morning, and evening); and d) Treatment group-3 (P3): the wound was treated with 10% celery leaf extract cream and then covered with gauze (twice a day, morning, and evening).

The wound treatment was performed for 14 days. Wound healing in white rats was observed by measuring the average length of the incision wound every day starting from the first day of wounding until day 14, according to the length of the normal wound healing process. After 14 days, the rats were euthanized by inhalation of excess technical chloroform.

### *Histopathological observation*

Histopathology was performed to examine tissue samples under a microscope. Histopathological tests were performed macroscopically to examine fibroblast growth. The procedure began with a 2 × 2 cm biopsy of the rat skin on days 5 and 14. Skin tissue was cut transversely to determine the number of fibroblast cells. Wistar rat skin tissue was stored in urine pots, soaked in 10% formalin, and stained with hematoxylin and eosin (H&E) at the University of North Sumatra Microbiology Laboratory. Crust formation, re-epithelialization, collagen fibers, angiogenesis, inflammatory cells, and fibroblast cells were observed and counted by digital analysis method, photographed with a camera and microscope at 100x

magnification, photographed 4x and stored. Photographic analysis of the number of fibroblast cells was carried out using Image Raster 3 software. The fibroblast cell tissue that appeared in the histological examination was recorded as a red pixel by the software. Tissues with different colors were selected and recorded as other tissue pixels.

### Data analysis

Data were analyzed using SPSS. To test for differences between groups, one-way ANOVA was used ( $p < 0.05$ ). To analyse which treatment group is the most effective, Post Hoc Test with LSD technique was conducted.

## Results

Phytochemical test results showed that celery leaf extract contained secondary metabolite compounds. Its contents are flavonoids, saponins, and tannins. These compounds will work to accelerate the wound healing process on the backs of white Wistar rats. Researchers made macroscopic observations of rat wound healing by observing its progress every day and then measuring the length of the wound. Wound healing in white rats was assessed by measuring the average length of the wound every day from the first day of wounding until day 14.

Table 3. Average wound healing

Hari ke-	P0		P1		P2		P3	
	cm	%	cm	%	cm	%	cm	%
1	2	0	2	0	2	0	2	0
2	1.952	2.4	1.922	3.9	1.876	6.2	1.888	6
3	1.904	4.8	1.844	7.8	1.746	12.7	1.678	16.5
4	1.874	6.3	1.784	10.8	1.634	18.3	1.512	24.4
5	1.756	12.2	1.642	17.9	1.438	28.1	1.218	39.1
6	1.666	16.7	1.46	27	1.224	38.8	1.048	48
7	1.572	21.4	1.252	37.4	1.082	45.9	0.898	55.1
8	1.47	26.5	1.17	41.5	0.918	54.1	0.748	62.6
9	1.266	36.7	0.918	54.1	0.72	64	0.616	69.2
10	1.182	40.9	0.73	63.5	0.608	69.6	0.474	76.3
11	0.964	51.8	0.632	68.4	0.522	73.9	0.276	86.2
12	0.832	58.4	0.514	74.3	0.366	81.7	0.1	95
13	0.732	63.4	0.42	79	0.204	89.8	0.12	94
14	0.554	72.3	0.314	84.3	0.046	97.7	0	100

Based on the observations made in all groups, a process of wound healing was observed in Wistar white rats. There was a difference in the average percentage of healing between the groups. The average percentages of incision wound healing on day 14, group P0 72.3%, group P1 84.3%, group P2 97.7%, and 100% in group P3, respectively. Therefore, it can be concluded that group P3 had a higher healing rate than groups P0, P1, and P2.

The results of the normality test were carried out using the One-Sample Kolmogorov-Smirnov test, and a significance value of 0.200 was obtained for each group ( $p > 0.05$ ). Therefore, it can be concluded that the data are normally distributed. Based on the results of the homogeneity test using the Levene test, and the probability value obtained was 0.964 ( $p > 0.05$ ), it can be concluded that groups P0, P1, P2, and P3 come from populations that have the same variance, or the four groups are homogeneous. Next, a One-way Anova was conducted to test the significant effectiveness

between the trial groups. The test results showed that the resulting significance value was 0.000 ( $p < 0.05$ ). It can be concluded that there was a significant difference between the treatment groups. A post-hoc LSD test was conducted to analyze the difference in mean LDL levels between the groups. The results of the Post Hoc LSD test showed a significance value of 0.000 (less than 0.05), which means that there was a difference in the average between groups.

Histopathological observations were performed

Table 4. Post Hoc LSD results

Group		Mean difference	p
P1	Treatment 2	.00985	.000
	Treatment 3	.00985	.000
	Treatment 4	.00985	.000
P2	Treatment 1	.00985	.000
	Treatment 3	.00985	.000
	Treatment 4	.00985	.000
P3	Treatment 1	.00985	.000
	Treatment 2	.00985	.000
	Treatment 4	.00985	.000
P4	Treatment 1	.00985	.000
	Treatment 2	.00985	.000
	Treatment 3	.00985	.000

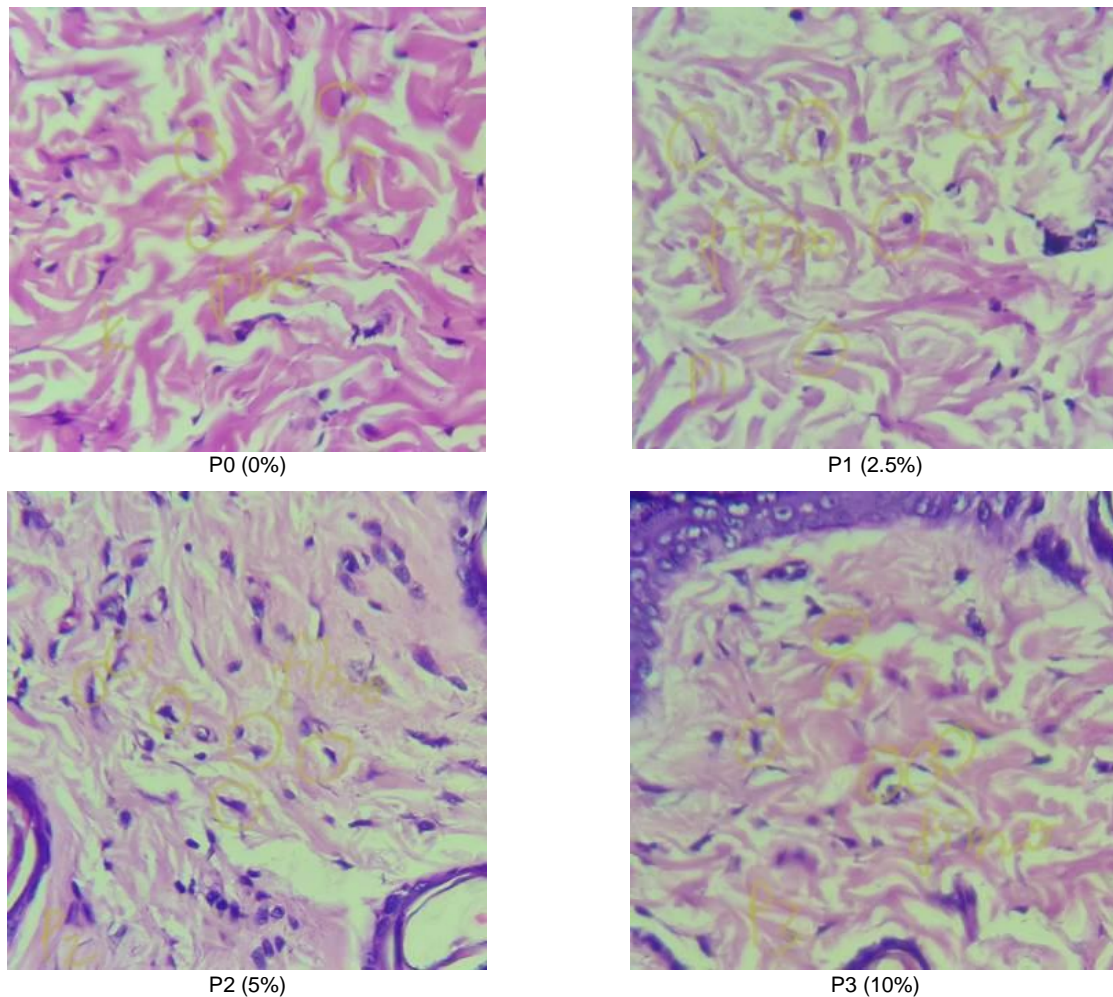


Figure 1. Observation of histopathological preparations (400x magnification)

using a light microscope at 400x magnification. The purpose of this study was to examine the structure and morphology of the cells, especially fibroblast cells, in each wound specimen in the treatment group with base cream (0), celery leaf extract cream at a concentration of 5%, celery leaf extract cream at a concentration of 2.5%, and celery leaf extract cream at a concentration of 10%. The cream was applied twice a day: in the morning and evening. Incision wound healing consists of several phases: the inflammatory, proliferation, and maturation phases. In the proliferation phase, fibroblasts play an important role in the production of proteins for wound healing, including collagen. The results of histopathological examination using a 400x magnification light microscope of incision wound healing showed differences in the number of fibroblasts.

In the P4 treatment group administered 10% celery leaf extract cream, the number of fibroblast cells was tighter than that in the P1 group given base cream without extract, P2 celery leaf extract concentration of 2.5%, and celery leaf extract concentration of 5%. In the treatment group given 10% celery leaf extract cream, fibroblast cells were many and rather tight compared to the treatment groups given base extract cream, 2.5% celery leaf extract cream and 5% which had fewer fibroblast cells and sparse distribution.

## Discussion

The purpose of this study was to examine the effect of topical application of a celery leaf extract cream preparation on the acceleration of the wound healing process on the surface of rat skin. In the initial phenomenon, it has been explained that normally, the wound undergoes a healing process and is a response of connective tissue that occurs in three phases that can overlap, namely the process of



inflammation (inflammatory phase), the process of building new tissue (proliferative phase), and the process of strengthening the tissue (maturation or remodeling phase).<sup>5</sup> Open wounds such as cuts are very susceptible to infection, especially by bacteria, and can be an entry point for systemic infections, therefore requiring wound care.

Natural materials, such as those used for wound care and healing, have also been widely used because plants generally contain flavonoids, saponins, and tannins that can act as wound healing agents. Celery is a plant that can play a role in wound healing. Celery contains flavonoids that can be used as antiallergic, anti-inflammatory, antiviral, anticarcinogenic and antioxidants that have the potential to prevent the formation of free radicals.<sup>16</sup> Flavonoids, saponins and tannins are also antioxidants, proangiogenesis and can increase oxygen supply and nourish injured skin.<sup>13</sup>

In the P4 treatment group administered 10% celery leaf extract cream, the number of fibroblast cells was more numerous and dense compared to the P1 group given base cream without extract, P2 celery leaf extract concentration of 2.5%, and celery leaf extract concentration of 5%. In the treatment group with 10% celery leaf extract cream, fibroblast cells were many and rather tight compared to the treatment groups given base extract cream, 2.5% celery leaf extract cream and 5% which had fewer fibroblast cells and sparse distribution. Group P4 (10% concentration) showed superior wound acceleration compared to the other groups. This can be seen from the average acceleration of healing faster than the other groups, and was the only group that experienced 100% healing. This can occur because celery leaf extract contains flavonoids, saponins, and tannins that can be used for their antiallergic, anti-inflammatory, antiviral, anticarcinogenic, and antioxidant properties, which have the potential to prevent the formation of free radicals. The results of this study are in line with previous research which concluded that celery extract cream has the activity of healing incision wounds in rabbits.<sup>16</sup> Another study concluded that the preparation of celery leaf ethyl acetate fraction cream has the activity of accelerating the healing of incision wounds to male white rats wistar strain.<sup>19</sup>

## Conclusion

The administration of bay leaf extract significantly reduced MDA levels, and based on the average MDA levels, it was observed that the group with a dose of 5 ml was superior compared to the other groups in reducing MDA levels in Wistar male white rats induced with excessive physical activity.

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