The skin plays an important role in the protection of the body's internal environment and is the largest organ in the human body, so serious damage to this organ can cause several problems in its survival. The skin consists of two layers of epidermis and dermis, which are placed over the subcutaneous adipose. The epidermis consists mainly of a layer of keratinocytes into which several other cell types have spread, including melanocytes and Langerhans cells. The epidermis is separated from the dermis by a basement membrane. The dermis is composed of papillary and reticular cells consisting of an extracellular matrix or a basal substance consisting of collagen, fibrous tissue, elastin, and glycosaminoglycans. Disruption of this wound-healing process will consume large resources and will often require long-term medical care.

**Effect of Administration of *Apium graveolens* (Linn.) Extract on the Number of Neutrophils, Angiogenesis, and Fibroblast Density Areas in Sprague Dawley Rats Incision Wounds**

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**A B S T R A C T**

**Background:** Wounds are a challenging clinical problem with complications that often lead to morbidity and mortality. The herbal plant *Apium graveolens* (Linn.) has anti-oxidant and anti-inflammatory effects, which are thought to accelerate the wound healing process. The number of neutrophils, angiogenesis, and the area of fibroblast density are elements that play an important role in the wound healing process. Thus, the administration of *Apium graveolens* (Linn.) extract is expected to assist in the wound healing process, which can be viewed from the number of neutrophils, angiogenesis, and the area of fibroblast density. **Methods:** An experimental in vivo study with a randomized post-test approach with a control group of rats that were given an incision on their back. Random allocation was performed to divide 20 Sprague Dawley rats into 4 groups. Neutrophil count, angiogenesis, and area of fibroblast density were seen histopathologically. Data were analyzed and processed using SPSS 25 software in univariate, bivariate, and multivariate ways. **Results:** The number of neutrophils, angiogenesis, and area of fibroblast density in the *Apium graveolens* (Linn.) extract group was lower than the group given the cream without *Apium graveolens* (Linn.) extract (p < 0.05), and there was no difference compared to the group given gentamicin cream. 0.1% (p > 0.05). Extract *Apium graveolens* (Linn.) had the lowest number of neutrophil and angiogenesis compared to the other groups (p < 0.05). **Conclusion:** The administration of *Apium graveolens* (Linn.) extract cream was effective in the incision wound healing process in terms of a decrease in the number of neutrophils, angiogenesis, and the area of fibroblast density. The administration of *Apium graveolens* (Linn.) extract cream was more effective than 50% extract cream.

**1. Introduction**

The skin plays an important role in the protection of the body's internal environment and is the largest organ in the human body, so serious damage to this organ can cause several problems in its survival. The skin consists of two layers of epidermis and dermis, which are placed over the subcutaneous adipose. The epidermis consists mainly of a layer of keratinocytes into which several other cell types have spread, including melanocytes and Langerhans cells. The epidermis is separated from the dermis by a basement membrane. The dermis is composed of papillary and reticular cells consisting of an extracellular matrix or a basal substance consisting of collagen, fibrous tissue, elastin, and glycosaminoglycans. Disruption of this wound-healing process will consume large resources and will often require long-term medical care.
management. Serious and extensive damage to the skin, such as burns, threatens the survival of the organism and impairs the regenerating capacity of the skin. In addition, with the increasing prevalence of diseases such as diabetes, vascular disease, and obesity, chronic wounds are becoming a major global problem with limited treatment options, unsatisfactory therapeutic effects, and high medical costs.4,5

Indonesia is a country with the second largest biological wealth in the world after Brazil. The potential for biological wealth makes Indonesia a great opportunity for the development of new modality therapy for wound management. *Apium graveolens* is one of the plants that are often found in Indonesia and is a plant with extraordinary medicinal potential. This plant has been used for generations to treat various health problems such as constipation, diarrhea, and skin health. *Apium graveolens* is rich in primary metabolites, secondary metabolites, various vitamins, and minerals. The rich content of various metabolites, vitamins, and minerals makes *Apium graveolens* rich in benefits. *Apium graveolens* is rich in caffeic acid, coumaric acid, ferulic acid, apigenin, luteolin, tannin, saponins, and kaempferol compounds. Apigenin, luteolin, and tannins have potent anti-inflammatory effects, which have the potential to activate various growth factors and chemokines and initiate angiogenesis processes that play a major role in wound healing.6-10 This study is the first study that seeks to explore the extract of *Apium graveolens* in the wound healing process through the exploration of the ability of *Apium graveolens* on neutrophil cells, angiogenesis, and fibroblast density in vivo.

2. Methods

This study is an experimental study with a post-test-only approach with a control group design. A total of 20 rats (*Sprague dawley*) Wistar strain was included in this study and met the inclusion criteria in the form of the male gender, weighing between 150-200 grams, and after 8-10 weeks. First, the rats were acclimatized for 7 days, then divided into 4 groups randomly, where each group consisted of 5 Rats. The group of rats was given topical cream without *Apium graveolens* (*Linn.*) (K1) extract, 0.1% gentamicin topical cream (K2), topical cream with *Apium graveolens* (*Linn.*) 50% (P1), and topical cream with extracts. *Apium graveolens* (*Linn.*) 70% (P2). The treatment was given for 7 days. This study has been approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Diponegoro, with the number No.129/EC/H/FK-UNDIP/XI 2018.

Induction of the excision wound was carried out by anesthesia in the rats using ketamine (dose of 0.015 mg/dose). gBW) intramuscularly and chlorate (0.0025 mg/gBW) subcutaneously. Shave clean the hair in the area of the dorsum of the mouse, and mark the area where the wound will be made (in this case, on the back of the mouse), with a diameter of 1 centimeter.

Rats were monitored daily for signs of distress and signs of infection. On the seventh day, the fistula tissue was terminated at the same time: The rats were anesthetized with a mixture of Ketamine-Xylazine (Ketamine dose 80mg/kgBW; Xylazine dose 10mg/kgBW) intraperitoneally and the skin and subcutaneous tissue were cut with a scalpel with a size of 1 cm x 1cm x 1cm. The tissue pieces were put into a buffered formalin solution (10% formalin solution in sodium acetate buffer until it reached a pH of 7.0). Then do fixation for 18-24 hours. After fixation, the tissue was then put into aquadest for 1 hour so that the fixation solution was lost. The tissue pieces were then put in graded concentration alcohol, alcohol-xylol solution for 1 hour, and then pure xylol solution for 2 x 2 hours. The tissue was then put into a liquid paraffin solution for 2 x 2 hours, after which the tissue was embedded in solid paraffin, having a melting point of 56-58°C. The tissue was cut about 6 m and glued onto a glass object that had been previously smeared with polylysine. The object glass was then heated in an incubator at a temperature of 56-58°C until the paraffin melted.

The next step is to stain the tissue with hematoxylin-eosin. The tissue was put into xylol I and II preparations for 5 minutes each. Rehydrate using absolute alcohol for 3x2 minutes. The preparation was
washed with running water for 2 minutes. The preparation was put in hematocycline-eosin (Lilie-Mayer) for 5 minutes and rinsed with running water for 2 minutes. The preparation was differentiated with 0.6% HCl for 1-2 dips and rinsed with running water for 2 minutes. The preparation is immersed in a saturated lithium carbonate solution about 2-3 times and rinsed with running water for 2 minutes. If the color is not blue enough, the preparation can be put back into the H&E solution for 2 minutes and then rinsed with running water. The preparation was immersed in eosin for 3 minutes. Dehydrated using 70% alcohol for 3x3 minutes for each concentration. Clearing with xylol I and II, dripped 1-2 drops of entelan. The preparation is closed with a cover slip.

Furthermore, the histopathological assessment of neutrophils, angiogenesis, and fibroblast thickness was carried out by Anatomical Pathologists with the help of ImageJ software.

After the data is collected, data cleaning, coding, and tabulation are carried out. All results were assessed by means ± standard deviation accompanied by normality test (Shapiro Wilk) and data homogeneity test (Levene Statistic). The test used in this research is One Way ANOVA, followed by Post-Hoc Test to assess differences between groups. The results are said to be meaningful if p ≤ 0.05. Data analysis was performed using SPSS version 25 for Windows.

### 3. Results

The lowest number of neutrophils was seen in the group given topical cream with *Apium graveolens* (Linn.) extract, namely 18.80 ± 9.25, while the highest number of neutrophils was seen in the group given topical cream without *Apium graveolens* (Linn.) extract which is 60.10 ± 9.12. Group P1 (getting *Apium graveolens* 50% cream) did not show a significant difference compared to K2 (getting gentamicin). Meanwhile, group P2 (getting *Apium graveolens* 70% cream) showed a significant effect in reducing the number of neutrophils compared to group K2 (getting gentamicin).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>60.10 ± 9.12</td>
</tr>
<tr>
<td>K2</td>
<td>27.56 ± 4.47</td>
</tr>
<tr>
<td>P1</td>
<td>24.88 ± 6.43</td>
</tr>
<tr>
<td>P2</td>
<td>18.80 ± 9.25*</td>
</tr>
</tbody>
</table>

*post hoc, Bonferroni VS K2, p<0.05.

The highest angiogenesis was seen in the group given topical cream without *Apium graveolens* (Linn.), which was 80.30 ± 9.66, while the lowest angiogenesis was seen in the group given topical cream with *Apium graveolens* (Linn.) 70%, i.e., 51.80 ± 11.68.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>80.30 ± 9.66</td>
</tr>
<tr>
<td>K2</td>
<td>58.80 ± 6.03</td>
</tr>
<tr>
<td>P1</td>
<td>57.70 ± 8.17</td>
</tr>
<tr>
<td>P2</td>
<td>51.80 ± 11.68</td>
</tr>
</tbody>
</table>

*post hoc, Bonferroni VS K2, p<0.05.
The highest mean area of fibroblast density was seen in the group given topical cream without *Apium graveolens* (Linn.) extract, which was 5.641±0.491, while the lowest mean area of fibroblast density was seen in the group given topical cream with *Apium graveolens* (Linn.) 70 %, i.e., 4.008±0.934.

Table 3. Comparison of fibroblast density area (in units of µm²) between groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K1</td>
<td>5.641±0.491</td>
</tr>
<tr>
<td>K2</td>
<td>4.538±0.817</td>
</tr>
<tr>
<td>P1</td>
<td>4.040±0.649</td>
</tr>
<tr>
<td>P2</td>
<td>4.008±0.934</td>
</tr>
</tbody>
</table>

*post hoc, Bonferroni VS K2, p<0.05.

The macroscopic appearance of the incision wound from Figure 1 shows that on the seventh day, the group that was given *Apium graveolens* (Linn.) had the best macroscopic appearance compared to the other groups.

4. Discussion

Phytochemical compounds *Apium graveolens* (Linn.) consist of carbohydrates, phenols (flavonoids), alkaloids, steroids, limonene, selenin, prokoumarin glycosides, flavonoids, Vitamins A and C make this plant often used in various traditional medicines. In addition, *Apium graveolens* (Linn.) contain a lot of phenolic acids such as caffeic acid, p-coumaric acid, and ferulic acid. The flavonoid content *Apium graveolens* (Linn.) consists of apigenin, luteolin and kaempferol. Flavonoids and many other phenolic components have been reported to be effective as anti-
oxidants, anticancer, antibacterial, cardioprotective, anti-inflammatory, and immune system agents, as well as promoting and protecting the skin from UV radiation. The anti-oxidant and anti-inflammatory role of *Apium graveolens* (Linn.) can help in the wound healing process.\textsuperscript{11-13}

The skin wound healing process is normal. The inflammatory phase usually lasts 2-5 days and stops once the noxious stimuli have disappeared. Neutrophils are first drawn to the injured area within 24-36 hours of injury. Neutrophils are part of the inflammatory response and can secrete signals that amplify inflammation in the early stages of healing. And also act as signals to close the inflammatory phase and are the first inflammatory cells to move to the wound site as a defense against infection. In the normal wound healing process, neutrophils undergo apoptosis which is eventually engulfed by macrophages, and the uptake of apoptotic cells by macrophages provides a strong signal for the resolution of inflammation, allowing the wound to proceed to the healing phase.\textsuperscript{14-17}

Angiogenesis is an important factor in the ability of tissues to repair themselves and is essential for removing debris and providing nutrients and oxygen to the wound layers. The formation of granulation tissue which is a dense network of blood vessels, macrophages, and fibroblasts embedded in a loose matrix of fibronectin, hyaluronic acid, and collagen, depends on the vascularity of the wound tissue and begins to appear in the wound about four days after injury. During the formation of granulation tissue, new blood vessels develop from pre-existing vessels. Angiogenic factors are secreted by fibroblasts, macrophages, keratinocytes, and endothelial cells themselves. In the proliferative phase, blood vessels in the base of the wound are estimated to increase fivefold to meet the metabolic demands of tissue-repairing cells. Progress toward the remodeling phase results in a significant reduction in the metabolic requirements of the new tissue. In this phase, there is a decrease in angiogenic molecules and an increase in angiostatic molecules that promote vascular regression. Neovascular return to a vascular density similar to the pre-injury state. Reduction of blood vessels in the wound tissue is necessary to increase the strength of the newly regenerated tissue and reduce scarring. After the injury, PDGF and TGF-\beta will stimulate fibroblast migration to the wound area. Fibroblasts will turn into myofibroblasts which will cause contractions at the edges of the wound, resulting in the approach of the wound edges, which can accelerate wound healing.\textsuperscript{18-20}

5. Conclusion

The administration of *Apium graveolens* (Linn.) extract cream was effective in the incision wound healing process in terms of a decrease in the number of neutrophils, angiogenesis, and the area of fibroblast density. The administration of *Apium graveolens* (Linn.) extract cream was more effective than 50% extract cream.

6. References


