Healing Capacity of Leaves Stalks Extract of *Eurycoma longifolia* Jack.) on Incised Wounds of Mice Skin

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**ABSTRACT**

**Introduction:** *Pasak bumi* is a medicinal plant commonly used as herbal medicine by Indonesian people. This study aims to examine the healing capacity of mice skin incised wounds by using *pasak bumi* leaves stalks ethanol extract ointment. **Materials and Methods:** This study used twenty-five male mice Balb/c strains, grouped into five groups, divided into three treatment groups (ointment with 20% extract concentration, ointment with 35% extract concentration, and ointment with 50% extract concentration), a positive control group using Betadine®, and the negative the negative control group using an ointment base. The back of the mouse was slashed 1.0 cm long with a depth of 0.2 cm. Then, the wound was smeared twice a day for 21 consecutive days. **Results:** The application of 20%, 35%, and 50% of ointment extract had the effect of healing the incised wounds after 21 days just as Betadine®. From the description score of the wound on the twenty-first day, it can be seen that the applied ointment with 35% of extract concentration shows the same healing capacity as Betadine® (p < 0.05), unlike the administration of ointment base (p <0.05). **Conclusions:** It can be concluded that *pasak bumi* leaves stalks has the potential effect to heal incised wounds.

**Key words:** *Eurycoma longifolia* Jack., Incised wound, Ointment.

**INTRODUCTION**

Wounds are the effect of tissue damage to the skin caused by contact with heat sources (such as chemicals, hot water, fire, radiation, and electricity), as the result of medical action or a change in physiological conditions. Based on the mechanism, there are six types of wounds: incised wounds, contusion wounds, broken wounds, leakage wounds, scars, and burns. Incised wounds are cuts caused by sharp-edged instruments. A contusion wound is the type of injury caused by a collision of the body with a blunt object which causes damage to the internal part of the body. A broken wound is the type of wound that has irregular, jagged contours and is quite deep. A leakage wound is a type of wound that causes a small hole in the surface of the skin but penetrates the body deeply. A scar is a type of wound which is not too deep but has a very wide wound surface and generally comes from the scratching of the skin on a rough surface. A burn is a type of injury caused by the burning of body parts. Injuries are a torn condition or a tearing of tissue in the body, whether it is on the skin, muscles, nerves, blood vessels, or lymph. There are various causes of injuries; among them are accidents, operations, radiation, electric shock, and chemicals. The wound healing process involves many cells in the body, through the stages of inflammation, proliferation, and maturation. Inflammatory stages begin at the time of injury and last up to two to three days, and if there is no infection in the inflammatory phase, the wound healing process will enter the proliferative stage. The proliferative stage starts on the second or third day and lasts until the third week. The next stage is maturation; this stage requires the longest time in wound healing.

Several factors that influence the wound healing process are age, nutrition, infection, hematoma, ischemia, diabetes, wound condition, and medication. Infection is an inhibiting factor in wound healing because it can cause inflammation and tissue damage. The signs of the inflammatory process are erythema (redness), oedema (swelling), heat, and the presence of pain.

Damage to the outer surface of the body will be the entry point for foreign objects. Therefore, an attempt is necessary for the body to heal wounds. According to Maryunzii, the wound healing process is divided into three phases: inflammation, proliferation, and maturation. The inflammatory phase is characterized by an increase in blood flow to the wound area and is followed by an increase in fibrin flow and white blood cells that work to eat microorganisms and dead cells. The proliferative phase is characterized by the formation of wound healing layers, and the final phase in maturation is characterized by a more apparent wound healing, resembles tissue and unification of the new collagen and suppresses the blood vessel that causes the wound to be flat and thin. Correspondingly, the result of the research showed that after five days, the wound would experience healing which was characterized by the formation of a scab that covers the entire wound area. This healing time is faster than normal because the wound is smeared with *tapak dara* leaf.

*Pasak bumi* is one of the native plants of Indonesia. Traditionally, all parts of this plant are being used as a herbal medicine. The *pasak bumi* root is commonly...
used as an aphrodisiac, but it is also used to treat dysentery, fever, and malaria. The bark and stems of the *pasak bumi* are useful to treat a fever, cancer sores, intestinal worms, bone pain, and tonic after childbirth. The leaves of the *pasak bumi* are useful for treating itching, while the flowers and fruits are used to treat headaches, abdominal pain, and bone pain.7

In addition, the stem of *pasak bumi* in medicine serves as a natural aphrodisiac for men.8 Moreover, the roots of *pasak bumi* have also been scientifically tested as an anti-malarial, 9-15 anti-bacterial,14 cytotoxic,12 anti-osteoporosis,13,16 anti-oxidants, and anti-inflammatory,17 hepatoprotector,18 and is also safe on the liver organ of lactating mice,19 while maintaining the physiological conditions of the lactating mice.20

The *pasak bumi* root contains various compounds including the canthin-6-one alkaloid, alkaloid β-carboline,11-12 quassioinds,12-21 triterpenic tirucallane-type,12 biphenylneolignan,12 and squalene derivatives.22,23 In connection with various potentials of *pasak bumi* roots, especially as an anti-inflammatory agent together with its various compounds, there are possibilities that other parts of this plant, especially the petiole, also have potential as medicine.

The investigative study of *pasak bumi* leaf stalks in medicine has not yet been reported. Some plants that were reported to have efficacy as incised wound healers are *Aloe vera* leaves,24 pisang ambon pseudostem,25 and binahong leaves.26 Furthermore, the result of phytochemical studies shows that *Aloe vera* leaves contain alkaloids, flavonoids, and steroids.27 The *pisang ambon* tree stems were reported to contain saponins, anthraquinones, quinones, and lignin.28 While *binahong* leaf stems contain alkaloids compounds, flavonoids, and tannins.29 Based on the above description, this study was conducted to examine the activity of incised wound healing capacity of *pasak bumi* (*Eurycoma longifolia* Jack.) leaves stalks ethanol extract.

**MATERIALS AND METHODS**

Animal and ethical approval

The experimental animals used were 25 male Balb/c mice aged 2-3 months with body weight ranging from 22-34 grams. Before treatment started, the animals were acclimatized for seven consecutive days by being fed and given water in *ad libitum*. The use of experimental animals in this test is under the ethical feasibility number 91 / II / HREC / 2018 issued by Sebelas Maret University in the Faculty of Veterinary Medicine, Indonesia.

**Extraction**

A total of 600 grams of *pasak bumi* leaves stalks including the stems and leaves were obtained from Sulai forest and Sungai Kerasi forest in Mensasak Hamlet, Hulu Gurung Subdistrict, Kapuas Hulu Regency and in Gunung Peramas, Kayong Utara Regency. The *pasak bumi* leaves stalks were cleaned was cleaned and then dried, then extracted by maceration using 70% ethanol. The maceration process refers to Harborne.30 Maceration was carried out for 3x24 hours twice. The resulting filtrate was evaporated, and a product of 16.76 grams was obtained.

**Qualitative phytochemical test**

Qualitative phytochemical tests carried out refer to Harborne.30 Phytochemical test results were reported by the Chemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjungpura University, Pontianak on certificate No. 038 / LABKIM / XII / 2017.

**Ointment making**

The formula used in making the ointment base refers to Agoes.31

| Ingredient | Amount |
|------------|--------|
| Vaseline album | 85g |
| Ointment base | 100g |
| Extract | 1g |
| Ointment base | 4g |
| m.f. ointment | 5g |
| Extract | 1.75g |
| Ointment base | 3.25g |
| m.f. ointment | 5g |
| Extract | 2.5g |
| Ointment base | 2.5g |
| m.f. ointment | 5g |

**The healing capacity of *pasak bumi* leaves stalks ethanol extract on incised wounds**

Mice were taken randomly and divided into five groups, where each group consisted of five animals. The hair on the back area was shaved and removed, and then the back area was cleaned with 70% alcoholic cotton, then the cut was done along 1 cm with a depth of 0.2 cm. The day the wound was made was stated as the first day. Furthermore, the wound in the first group (the positive control group) was smeared with Betadine®, the second group was smeared with an ointment base (negative control group), and the third group was smeared with *pasak bumi* leaves stalks ethanol extract ointment (extract concentrations of 25%, 35% and 50%). The ointment was applied by applying a thin layer evenly on the surface of the wound. Basting was done twice a day at 08.00 a.m and 02.00 p.m for 21 days. During those days, observations and scores were determined based on the description of the wounds from each individual.

**Data analysis**

This study used a completely randomized design. Wound scores on the second, fourth, seventh, fourteenth, and twenty-first days were statistically analyzed and if they were significantly different (p <0.05) followed by Duncan’s test. Data analysis was performed using SPSS version 25.

**RESULTS AND DISCUSSION**

There were some limitations on this research including the wound that tested is only the wound that is made by the procedures, the *pasak bumi* ointment made are limited to the concentration as made; and the observation is limited to descriptive only. Regarding the time needed for the wound healing, the results of this study indicate that by applying *pasak bumi* leaves stalks ethanol extract ointment requires a shorter time for healing. Moreover, *pasak bumi* leaves stalks ethanol extract ointment has a similar healing capacity to cure incised wounds as well as Betadine® (Figure 1., Table 1., and Table 2.).
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Figure 1: Changes of incised wound based on the 2nd, 4th, 7th, 14th and 21st day.

Table 1: Incised Wound Scores.

| Score | Wound Description |
|-------|-------------------|
| 1     | The wound closes perfectly; there is no visible scar and hair is covered. |
| 2     | The wound closes, the scar starts to fade, and the hair begins to grow. |
| 3     | The wound closes, but there is a scar, and the hair begins to grow. |
| 4     | The wound closes, but there is a scar, and it is not covered with hair. |
| 5     | The wound closes, and there is no scab. |
| 6     | The wound closes, and the scab begins to disappear. |
| 7     | The wound closes, but there is still a scab, and the red colour around the wound is gone. |
| 8     | The tip of the wound begins to close, the scab is formed, and the colour around the wound is still pale red. |
| 9     | The tip of the wound begins to close, the edges of the wound begin to dry and harden, and the colour around the wound is still pale red. |
| 10    | The length of the wound is 1 cm, the edges of the wound begin to dry out, and the red colour of the wound and surrounding area decreases. |
| 11    | The length of the wound is 1 cm, the edges of the wound begin to dry out, the red colour of the wound and the surrounding area is reduced, and there is no infection detected. |
| 12    | The length of the wound is 1 cm, the wound is still wet, and the red colour of the wound and the surrounding area is reduced. |
| 13    | The length of the wound is 1 cm, the wound is still wet, the colour of the wound and the surrounding area is red and infection detected. |
| 14    | The length of the wound is 1 cm, the wound is still wet, and the colour of the wound and the surrounding area is red. |

Table 2: Average score of incised wound based on the description of the wound. Observations were made on the 2nd, 4th, 7th, 14th and 21st day.

| Day   | K1       | K2       | K3       | K4       | K5       |
|-------|----------|----------|----------|----------|----------|
| 2     | 14.00 ± 0.00 | 12.00 ± 0.00 | 11.60 ± 0.89 | 10.80 ± 1.09 | 12.40 ± 0.54 |
| 4     | 10.00 ± 0.00 | 11.60 ± 0.89 | 10.40 ± 0.89 | 10.40 ± 0.89 | 10.60 ± 0.54 |
| 7     | 9.40 ± 0.54 | 9.40 ± 1.94 | 9.60 ± 0.54 | 8.80 ± 0.83 | 9.40 ± 0.54 |
| 14    | 4.80 ± 1.09 | 5.50 ± 1.91 | 4.00 ± 0.00 | 4.00 ± 1.41 | 7.80 ± 0.44 |
| 21    | 1.20 ± 0.44 | 1.50 ± 0.57 | 1.20 ± 0.44 | 1.40 ± 0.54 | 3.80 ± 1.48 |

Different letters on the same line show significant differences in the Duncan test (p <0.05). *The number shown after the symbol ± shows the standard deviation (SD) value. K1: Betadine®; K2: pasak bumi leaves stalks ethanol extract ointment 20%; K3: pasak bumi leaves stalks ethanol extract ointment 35%; K4: pasak bumi leaves stalks ethanol extract ointment 50%; K5: ointment base.
From the results of observation and scoring, it was known that on the second day, the condition of the wound in each treatment group was varied. Even though overall observation showed that the wound was still red and had not closed, the wound score was still high. However, compared to other groups, the condition of the wounds for groups in which the researchers applied the pasak bumi leaves stalks ethanol extract ointment 20%, 35%, and 50% was more improved; even the condition of the wounds on the application of pasak bumi leaves stalks ethanol extract ointment had reached the best result (p <0.05). While the observation on the fourth day showed the wound had begun to dry and the red color on the wound had begun to decrease, on the contrary, in the group with the administration of the extract pasak bumi leaf stalks ethanol 20% was still wet around the wound (p <0.05). This is in line with the opinion of Maryunani\textsuperscript{3} who said that the inflammatory process begins after surgery and ends on the third day or sometimes the fourth day and Sewta et al.\textsuperscript{24} who states that by giving Aloe vera leaf extract on the third day the wound begins to dry out and the length of the wound is also reduced.

The observation on the seventh day showed that the condition of the wound in the whole group was improved and it was marked by the tip of the wound, which began to close; the edge of the wound had begun to dry and harden, and the colour around the wound was pale red. Furthermore, observation on the fourteenth day showed that wounds cured by Betadine\textsuperscript{®} and the pasak bumi leaves stalks ethanol extract had closed while the scab had disappeared; otherwise, the wound with the ointment base was still marked by scab and pale red around the wound. In line with the result of research by Sewta et al.\textsuperscript{24} on the fourteenth day after the wound was given the aloe vera leaf extract, it had closed properly. According to Kanazawa et al.\textsuperscript{23} on the fourth, seventh, and fourteenth day, the wound healing process began to enter the proliferative stage. This stage is the stage of tissue formation and wound closure.

Furthermore, Dewi et al.\textsuperscript{5} stated that the process of granular tissue formation is a marker of healing, and this process starts from the bottom of the wound. The tissue formation begins with the growth of capillaries, and this granulation process goes along with the re-epithelialization process. In the final stage of this process, an epithelialization process and this granulation process goes along with the re-epithelialization formation is a mark of healing, and this process starts from the bottom of the wound. Furthermore, observation on the fourteenth day of this process, an epithelialization process will occur on the wound surface, and the wound will develop into a scab that consists of plasma and prodeni which mixes with dead cells. Moreover, Harvey\textsuperscript{31} states that the re-epithelialization process shows the phase when the wound has been closed by epithelial cells. Macrophages release epidermal growth factors that will stimulate the proliferation and migration of epithelial cells. Epithelial cells can only move up the active tissue and require a moist environment. Regarding humidity regulation, Yanhendri\textsuperscript{66} stated that to preserve the wound from infection and to moisturize the skin, the ointment can be used as a semisolid preparation made from fat, which is often used for local treatment of the skin.

The result of observation on the twenty-first day showed that wound healing began to enter the maturation or matured stage. The condition of the wound in the group with the administration of Betadine\textsuperscript{®} and pasak bumi leaves stalks ethanol extract had closed correctly, there were no scars and the skin had been covered with hair. The group with the ointment base had closed, but there was still a scar and the hair just began to grow (p <0.05). Dewi et al.\textsuperscript{5} stated that at the maturation stage, collagen fibbers will increase gradually and will get thicker. Increased collagen fibbers will cause the number of leukocytes to return to the normal range. Furthermore, collagen fibbers will spread by bonding together gradually to support tissue recovery.

Overall, from the results of this study, it was found that pasak bumi leaves stalks ethanol extract have anti-inflammatory capacity while healing wounds. This capacity is assumed to be related to the content of alkaloids, terpenoids, saponins, tannins, and phenols in the pasak bumi leaves stalks ethanol extract. As a result of previous studies, Kimura et al.\textsuperscript{36} reported that saponin compounds could trigger Vascular Endothelial Growth Factor (VEGF) and increase the number of macrophages to migrate to the wound area. This process can increase the production of cytokines to activate fibroblasts in wound tissue. In addition, Komboj and Saluja\textsuperscript{37} and Hidayati and Harjono\textsuperscript{38} each reported that terpenoid and alkaloid act as anti-bacterial. In addition, Ashok and Upadhyaya\textsuperscript{39} stated that tannins act as astringents, substances that are useful for shrinking the wound so that bleeding can stop faster and the wound dries more quickly. The tannin, terpenoid, and alkaloid compounds act as an astringent or as a bleeding stopper, overcoming the inflammation, accelerating wound healing and increasing new tissue regeneration\textsuperscript{40} while phenol plays a role in helping wound healing process with collagen formation.\textsuperscript{41} In the near future, we would try to explore the other part of this plant to find its function as a wound-healing agent.

CONCLUSION

The conclusion of this research the pasak bumi leaves stalks ethanol extract ointment 35% has proven to have the best healing capacity for the incised wound, in comparison to another composition of extract, and it is similar to the Betadine\textsuperscript{®} as a positive control.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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**Graphical Abstract**

**Pasak Bumi (Eurycoma longifolia Jack.)**

**Ointment**

**Heal incised wounds of mice skin**

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