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Burns Inoculated with *Staphylococcus aureus* Healed by Leaf Sheath Extract of Pisang Ambon (*Musa acuminata*)

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ABSTRACT

Objective: Using parts of banana to cure diseases is a common practice for people in homeland of genus *Musa*. However, scientific researches on biomedical effects of a specific banana cultivar still leaves many questions. This study is intended to find out the healing effect of leaf sheath extract of pisang Ambon or Gros Michel banana, a non-Cavendish cultivar of *Musa acuminata*, on burn wound intentionally inoculated with *Staphylococcus aureus*. Eighteen male rabbits were divided into six groups: P0, P1, P2, P3, P4, and P5 each consisting of three rabbits. P0 is rabbits that received no treatment as the negative control; P1, P2, P3 and P4 respectively are received banana extract of 6.25%, 12.5%, 55%, and 50%; P5 are given bioplaconton ointment as the positive control. To inflict burn wound, the withers area on the animal's back were shaved and antisepted using 70% ethanol and 10% iodine. On the shaved skin, burns was made by placing a heated brass coin weighted 10 g and a diameter of 2 cm, at 200°C for 5 seconds. All treatments were given topically three times daily. The recovery of burns is measured every three days for 14 days and the healing criteria were based on wound diameter changes. The results showed the extracts of pisang Ambon at the concentration of 12.5% and 25% are able to totally heal the wound on the day 14. While extracts of 6.25 % and 50% has not provided a complete recovery on day 14 but still give a significant healing in comparison to negative control. Thus, leaf sheath extract of Gros Michel banana is effectively heal burns infected by *S. aureus*.
Keywords: Pisang Ambon, Gros Michel Banana, *Musa acuminata*, *Staphylococcus aureus*, Burns, Wound Healing

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Introduction

Among the few types of versatile plants, banana is one of them. For people in homeland of the genus *Musa* banana is more than edible plant because all parts of the plant are useful. In Southeast Asian region for example, banana may be used for food, fodder, medicine, stimulant, household, shelter, cordage, clothing, textiles, ornamental, ceremonial, magic and ritual [1]. Pisang Ambon is the Indonesian name for Gros Michel banana, a non-Cavendish cultivar of *Musa acuminata* [2]. In Indonesia, pisang Ambon planted mainly for its fruit. However, as other types of banana as well, all parts of the Gros Michel banana are also used for a variety of purposes. Leaves, for example, used for wrapping food or dishes, while the pseudo-stem is used for rope. In Indonesian folk medicine system, some parts of this plant are used for treating diarrhea, wound healing, antivenom, and antiinflammation or swelling [3, 4].

In the last three decades, mainly in the developing country, there were numerous scientific studies on therapeutic and medicinal effect of banana plants. The findings indicated that banana plant extract has potential effects on epilepsy, leprosy, fevers, hemorrhages, acute dysentery, stomach ulcer, diarrhea, blood glucose and cholesterol, urinary system, muscular system, cancer, migraine, hypertension, and enzyme system [5, 6].

Wound healing effects and antimicrobial activities of banana have also been investigated by many researchers. Methanol extract of *Musa paradisiaca*, for instance, was revealed to show healing effect against thermal wound in Wistar albino rats and antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Phytochemical of the banana that are thought to give effect on wound healing and anti-bacterial is glycosides, tannins and alkaloids, saponins, flavonoids and phenols [7]. While *Musa sapientum* is alleged to have a wound healing activity due to the ethanolic crude extract of this banana proven to heal wound in male Swiss mice [8]. Both types of banana, *Musa paradisiaca* and *Musa sapientum*, are known as the common herbs used for treating diarrhea, dysentery, ulcerative colitis, diabetes, sprue, uremia, nephritis, gout, hypertension and cardiac diseases [9].

Another species of banana that shows antimicrobial activities is *Musa acuminata*. The flower extract of this banana exhibit inhibitory effects against *Staphylococcus aureus*, *Proteus mirabilis*, *Bacillus subtilis*, *Aspergillus niger*, *Candida albicans*, *Micrococcus sp* and *Salmonella sp*. Phytochemicals of this cultivar that allegedly responsible for antimicrobial activities are glycosides, tannins, saponin, steroids, phenols, alkaloids, antraquinones, flavanoids, carbohydrates [10, 11].

Although aspects of phytochemical, pharmaceutical and medicinal of banana have been widely studied, but there are still questions that need to be answered, particularly in relation to antibacterial and wound-healing properties of the plants. Firstly, given the anti-microbial test of the banana plant extract is generally performed in vitro, then what is the effect if the test done in vivo? Secondly, given banana plant consists of many varieties and each variety may contain bioactives of different types and concentration, it is very likely the difference of banana cultivars give different biological effects. This study, then, designed to investigate healing effects of leaf sheath extract of Gros Michel banana on burns wound that intentionally inoculated with *Staphylococcus aureus*.

Materials and Methods

Plant Samples and Extraction

Plant samples of pisang Ambon (*Musa acuminata*) were collected from the Village Bandar Negeri, Sub-district of Labuhan Maringgai, the District of Lampung Timur, the Province of Lampung, Indonesia. Taxonomic identification of the plant was done by botanist at the Botany Laboratory, University of Lampung, Indonesia. Of the banana pseudo stem with a diameter of 25 cm and a length of 2 meters, 5 leaf sheaths were peeled off. The fresh leaf sheaths were washed with water and cut into small pieces and put into a flask for maceration. Maceration was done by soaking 2kg of the samples in 96% ethanol for 24 hours and stirred three times daily. After 24 hours, the mixture was filtered and the pulp was soaked further in 96% ethanol until the pulp no longer change the color of the solvent. After being filtered and free from the pulp, macerate then concentrated by rotary vacuum evaporator. The

yielded crude extracts diluted with double-distilled water into the appropriate concentration and ready for used.

Leaf sheaths are used in the experiment for two reasons. First, the leaf sheath, as the structural part of the pseudo stem, are the largest part of the banana plants that could potentially be a agricultural waste. By being utilized, the banana plant waste can provide added value. Secondly, leaf sheath of the banana is rich source of bioactive substances including minerals that are commonly needed in wound healing process [12].

Bacterial Isolates

Staphylococcus aureus strain used in this study were obtained from Lampung Provincial Health Laboratory. The bacteria were isolated from patients with wound containing pus with sterile cotton swab and incubated in nutrient agar (NA). For the purposes of this study the bacteria isolates were grown in nutrient agar (NA) slants incubated at 35° C for 24 hours. From the NA media a small amount of the bacteria were scraped off using steril loop and transferred to nutrient broth (NB). After 24 hours of incubation in NB medium, the isolates then diluted 10⁻³ times to make it ready for used in burn wound inoculation. The using of *S. aureus* was based on pathological nature of this bacteria that associated with several skin diseases such as boils, pimples, and make epidermal wound difficult to be healed [13]. Principally, this experiment refers to a similar study done by [14] to test the effect of inoculation of *S. aureus* on healing of burns induced by H₂SO₄.

Animals and Wound Infliction

Animals used in this study were male rabbits aged 4-5 months weighing 1-1.5 kg obtained from animal house of the Veterinary Centers of Lampung, Indonesia. Before treatment the rabbits were acclimatized for the experimental room for one week. Burn wound infliction procedure used in this study is adopted and modified from previous studies [15], [16], [17].

To inflict burn wound on rabbit skin, the withers area on the animal's back were shaved and antisepted using 70% ethanol and 10% iodine. On

the shaved skin burns was made by placing a brass coin weighted 10 g and a diameter of 2 cm that previously heated over the open flame for 5 seconds. Prior to and during burn wound creation all animals were anesthetized with chloroform using open mask method. Once the skin is blistered and open the wound was inoculated with the *Staphylococcus aureus* by dropping 0.1 ml of the diluted isolates. The animals were then housed individually in separate cages after complete recovery from anesthesia.

Wound Treatment and Healing Parameters

In this study, 18 rabbits were grouped into six consisted of three rabbits each, namely P₀, P₁, P₂, P₃, P₄, and P₅. P₀ is animals without treatment as the negative control; P₁ is group treated with 0.3 ml of banana leaf sheath extract of 6.25%; P₂ is group received 0.3 ml extract of 12.5%; P₃ is group given 0.3 ml extract of 25%; P₄ is rabbit group treated with 0.3 ml extract of 50%; and P₅ is rabbits that received bioplacenton ointment as the positive control. All topical treatments were conducted three times daily and wound recovery is observed and measured every three days for 14 days and the healing of burns was based on wound diameter changes.

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) and the difference among samples was determined by Least Significant Difference (LSD) Test at $\alpha < .05$.

Results and Discussion

The average of burn wound diameter on rabbit skin after treatment are presented in Table 1 and the healing pattern of each treatment was visualized in Figure 1.

Based on the data presented in the table and graph mentioned above, there was no treatment could heal the burns before 14 days. However, leaf sheath extracts of Gros Michel banana at the concentration of 12.5% and 25% on the day

14, are able to totally heal the wound (score of 0). Although banana leaf sheath extract of 6.25 % and 50% has not provided a complete recovery on day 14, but given results of the LSD test showed a significant difference with the control group, it can be said that leaf sheath extracts of the pisang Ambon were able to recover thermal wound that intentionally inoculated with *S. aureus*. Interestingly, the healing effect of leaf sheath extract of the banana was as effective as the standard drug of burns, bioplacenton.

The process of burn wound healing is similar to the general principles of the wound healing process [18]. Wound healing is a biological process consisting of four phases namely hemostasis, inflammation, proliferation, and remodeling. If there is factor interfere with one of the phases, wound healing by itself will be impaired [19]. Conversely, factors that can modulate biochemical process in the phases can be categorized as a wound healing agent. There are plant-derived substances that can function as the modulators of fibroblast and keratinocyte for example alkaloids, terpenoids, polyphenols; modulators of collagen synthesis for example flavonoids, terpenoids, tannins and other phenolic compounds; modulators of cytokines and growth factors for example alkaloids, flavonoids, tannins, terpenoids [20]. All of the phytochemicals needed for wound healing mentioned above are found in and can be extracted from banana plants including *Musa acuminata* species [21].

In this study, the burn wound created on rabbit skin was intentionally inoculated with *Staphylococcus aureus*. The healing of the burns, of course, can not be separated from the role of the bioactive compounds such as alkaloid, flavonoid, tannin, terpenoid and other phenolic compound in inhibiting bacterial growth [22, 23, 24, 25, 26]. Banana plants, especially *Musa acuminata*, a species in which Gros Michel cultivar grouped, is recognized as rich in alkaloid, flavonoid, terpenoid, coumarin, phenol, tannin, glycoside, steroid and saponin [27, 28].

In addition to affected by bioactive compounds such as alkaloids, flavonoids, tannins, terpenoids, wound healing is also depending on other type of essential nutrients including vitamins such as vitamin A, C, and E, and trace elements namely Cu, Mn, Zn, and Fe. Vitamin A

is required for cellular differentiation in epithelial and bone formation, vitamin C is necessary for collagen formation, while vitamin E is the major lipid-soluble antioxidant in the skin [29]. Copper is essential for collagen cross linkage, manganese is the component of enzymes needed for tissue regeneration, zinc is needed in fibroblast proliferation, and iron is component of many enzyme systems and plays a key role in promoting collagen synthesis and oxygen delivery to tissue [30]. Phytochemical screening of pseudo-stem of banana plants including *Musa acuminata* X *balbisiana* cv. Awak was found to contain Na, K, Ca, Mg, Si, Fe, Zn, Mn, and P [31, 32] and vitamins namely vitamin A and vitamin C [33].

Conclusion

The results of this study support the uses of banana in the folk medicine system practiced by people in homeland of the genus *Musa*. Thus, it can be conclude that leaf sheath extract of pisang Ambon or Gros Michel banana (*Musa acuminata*) is effectively healing burn wound that intentionally inoculated with *Staphylococcus aureus*.

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Conflict of Interest

Authors declare no conflict of interest.

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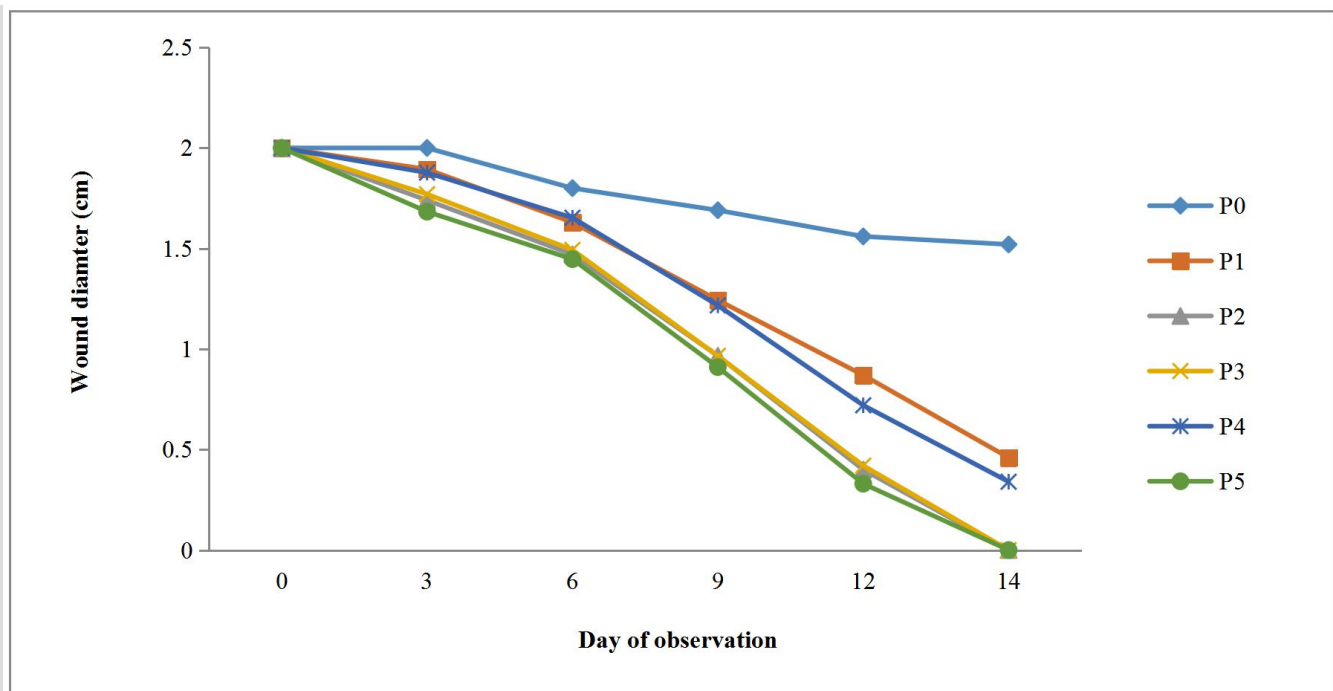


Figure 1. The healing pattern of burn wound on rabbit skin inoculated with *Staphylococcus aureus* after treatment. P₀ is rabbits without treatment as the negative control; P₁, P₂, P₃ and P₄ respectively are the rabbits that treated with banana leaf sheath extract of 6.25%, 12.5%, 25%, and 50%; P₅ is rabbits that received bioplacenton ointment as the positive control.

Table 1. Average score of burns healing according to day of assessment, the Anova and LSD test results

Treatments	Burn wound diameter on day...				
	3	6	9	12	14
P0	2 ^a	1.80 ^a	1.69 ^a	1.56 ^a	1.52 ^a
P1	1.893 ^b	1.63 ^b	1.243 ^b	0.87 ^b	0.46 ^b
P2	1.74 ^c	1.47 ^c	0.966 ^{bc}	0.40 ^d	0 ^e
P3	1.77 ^c	1.493 ^{cd}	0.966 ^{bc}	0.42 ^d	0 ^e
P4	1.877 ^b	1.653 ^b	1.217 ^{bc}	0.72 ^c	0.34 ^c
P5	1.683 ^d	1.447 ^d	0.916 ^d	0.33 ^e	0 ^e
F-test	59.5087 ^{**}	69.4545 ^{**}	121.1362 ^{**}	610.8491 ^{**}	2,560.78 ^{**}

P₀ is rabbits without treatment as the negative control; P₁, P₂, P₃ and P₄ respectively are the rabbits that treated with banana leaf sheath extract of 6.25%, 12.5%, 25%, and 50%; P₅ is rabbits that received bioplacenton ointment as the positive control. Values in the same column that share the same superscript are not different at $\alpha=0.05$. F-test values labeled ^{**} mean 'significantly different' at $\alpha = 0.01$.

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