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5

Effect of Saba Banana Peel Extract (*Musa paradisiaca* L) On Incision Wound Healing in Mice (*Mus musculus*)

Muhammad Harun Achmad¹, Muhammad Haritza Aqilah², Huldani³

¹Lecturer of Pediatric Dentistry, Department of Pediatric Dentistry Faculty of Dentistry, Hasanuddin University, Indonesia

²Clinical Student of Faculty of Dentistry, Hasanuddin University, Indonesia

³Department of Physiology, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia

Corresponding author: harunachmader@gmail.com

ABSTRACT

Background: Wounds are damage that occurs to the body caused by a myriad of destructive factors. When a wound arises, a wound healing process will occur which begins with the bleeding process. The wound healing phase is divided into 4 phases, namely the hemostatic phase, inflammation, proliferation and remodeling. There are several ingredients that can affect wound healing such as flavonoids and tannins. Flavonoids and Tannin can be found in high levels in Saba banana peel, so it has the potential to be used as an agent to accelerate the wound regeneration process after extraction process. The mechanism of action of flavonoids and Tannin in wound healing is to inhibit the formation of inflammatory mediators excessively so that the inflammatory process can take place normally and the healing process can be accelerated. **Research Objectives:** The purpose of this study was to determine the effect of Saba banana peel extract (*Musa paradisiaca* L.) on incision wound healing in mice (*Mus musculus*). **Research Methods:** This study was a laboratory experimental study with a post-test only control group design. The test animals used were 27 male mice divided into three treatment groups according to the specified inclusion and exclusion criteria. This study was measured by looking at the time to heal incision wounds until the wound has closed. The collected data was then processed using the SPSS version 24 program. **Results:** Based on observations, it was found that in the povidone iodine group and Saba banana peel extract, the wound healed within 9 days, whereas within 9 days there was no healing in the aquadest group. Based on the data analysis it was found that the average healing time in the povidone iodine group was 2 mm and the Saba banana peel extract was 1.88 mm. **Conclusion:** Saba banana peel extract (*Musa paradisiaca* L) can accelerate the healing time of incision wounds in mice. Suggestion for further research is to do histopathological examination so that the results of the study can be accurate.

Keywords: wound healing, incision wound, kepok banana skin, povidone iodine, aquadest, mice.

Introduction

Wounds are damage to the body that are caused by cuts, bludgeoning with an object with sharp or blunt edge. One of the types of wounds are incision wound, incision wound are defined as wounds that appeared because of a cut using sharp edged objects which in turn resulted in a narrow and long opening of the particular wound¹. During the wound creating, there will be a hemorrhagic occurrence. Under normal condition, when a wound is created then there will be hemorrhaging which would stop in 2-7 minutes and the wound will heal after going several phases of wound healing².

During the wound healing phase, the one that acts the most during the inflammation process and the body's capability to return to a hemostatic condition to create an optimum condition for wound healing and also the create a wound that is clean from microorganisms. Thus, it can be concluded that in order to heal a wound in a normal period of time, bodily conditions need to be in a hemostatic condition and free of microorganisms contamination². The period of wound healing can be influenced by several factors such as infection, age, drugs consumption and the cleanliness of the wound³.

A research done by Ilham et al discovered that the peel of Saba Banana has a beneficial usage. According to Dewanti, which were quoted by Ilham et al, the utilization of the banana peel is very minimal thus resulting in unused waste by the byproduct of banana consumption⁴. On a research done Ahmed Aboul-Enein et al, it was discovered that the peel of Saba Banana (*Musa paradisiaca L*) contains Flavonoid and Tannin⁵. Flavonoid and Tannin are Phenolic compounds. Flavonoid were commonly found on several plants such as God's Crown (*Phaleria macrocarpa*), aromatic ginger (*Kaemferiae galanga*), Tamarind (*Tamarindus indica*). A research done by Ramadhani N and Sumiwi S discovered that almost all of these plants has a similarity which is containing flavonoids⁶. Other than containing flavonoids, Saba Banana Peel also has a high Tannin contents thus the extract of the Saba Banana peel also has anti-microbial properties⁷. With both phenolic compounds being discovered, it can be concurred that the potential of Saba Banana peel to influence wound healing time positively, other than the anti-inflammatory capability of Flavonoids and also the anti-microbial properties of Tannin. Moreover, Saba Banana peel as a wound healing media can decrease the amount of production byproduct of commercial use of Saba Banana⁸.

Based on the explanation above, an interest sparked on the author in order to use the potential usage of Saba Banana peel as a medium that can influence wound healing, either accelerating it or slowing it down.

Research Method

The research will be conducted on Pharmacognosy, Phytochemistry and Pharmacology Laboratory of Faculty of Pharmacy, Pancasakti University, on Makassar in the month of April 2019 – until the needed result is received. This research uses *post test only control group design* model. The type of research done is experimental laboratory research. Samples used in this research are Mice (*Mus musculus*) in which an incisional wound will be created on the labial gingiva of the upper incisivus teeth.

Inclusion criteria of this research are, male mice, healthy, weighing 26-30 grams, aged 2-3 months and has been adapted to laboratory condition. Exclusion criteria in this research are mice that are incapable of adapting (refusal to eat, etc) and mice that died during the adaptation process.

The creation of Saba Banana peel extract (*Musa paradisiaca L.*) during the laboratory process is by maceration. Mice selection is done in accordance with sample criteria. Mice are placed in their containment place, and were fed and given water twice daily and were adapted to lab condition for 1 week. Mice were then divided into 3 groups at random. The next procedure were the creation of an incision wound on the upper incisivus labial gingiva with 2 mm of length and 1 mm thickness. On mice of

group 1, the extract of Saba Banana peel (*Musa paradisiaca*) were applied on the incision wound. On group 2 Povidone Iodine were applied on the wound and on group 3 sterile aquadest were added to the wound. An observation is then conducted clinically everyday since the wound creation until complete wound healing has been achieved.

The research were conducted during April – May 2019 Pharmacognosy, Phytochemistry and Pharmacology Laboratory of Faculty of Pharmacy, Pancasakti University, Makassar. The first thing done during the research is by extracting the researched substance, in this case the Saba Banana peel using maceration method. Next procedure is the adaptation procedure of the test animal with the lab condition. There were 27 mice which would be divided into 3 groups with each groups consisting of 9 mice. All 3 groups would receive different compounds for healing the incision wound, and then an observation is conducted. On group 1 or treatment group, the mice were given the Saba Banana peel extract. On group 2 or positive control were given Povidone Iodine. On group 3 or negative control, sterile aquadest were applied to the wound. The observation were done on each group until complete healing on the aforementioned group has been achieved, when it is achieved observation of said group is finished. Once all wound from all the groups have healed completely, data analysis were conducted and the process of synthesizing the research result can begin.

Data Analysis

During the research 27 male mice were used as sample, which were divided further into 3 groups of nine. Group 1 being the treatment group using the Saba Banana peel extract, 2 being the positive control using Povidone Iodine and 3 being the negative control using sterile aquadest. Descriptive analysis were done statistically using the research data. Data analysis of the treatment, are descriptively shown with Table 1, as follows :

Table 1. Descriptive Statistics

Treatment	Average	Deviation Standard	Error Standard
Extract	1,8833	0,08292	0,02764
Control	0,9056	0,06821	0,717

Table 1 shows descriptive result of the obtained data regarding the treatment using Saba Banana peel extract and control group. The obtained result of the extract has an average of 1,833 and for the control variable were 0,0956, moreover, the deviation standard of extract were found to be higher than those of the control group, this shows that the data distribution on extract were found to be better in comparison with the control variable. This is in line with Error Standard of the aforementioned data distribution which shows an error score that were found on the extract group were smaller in comparison with control group.

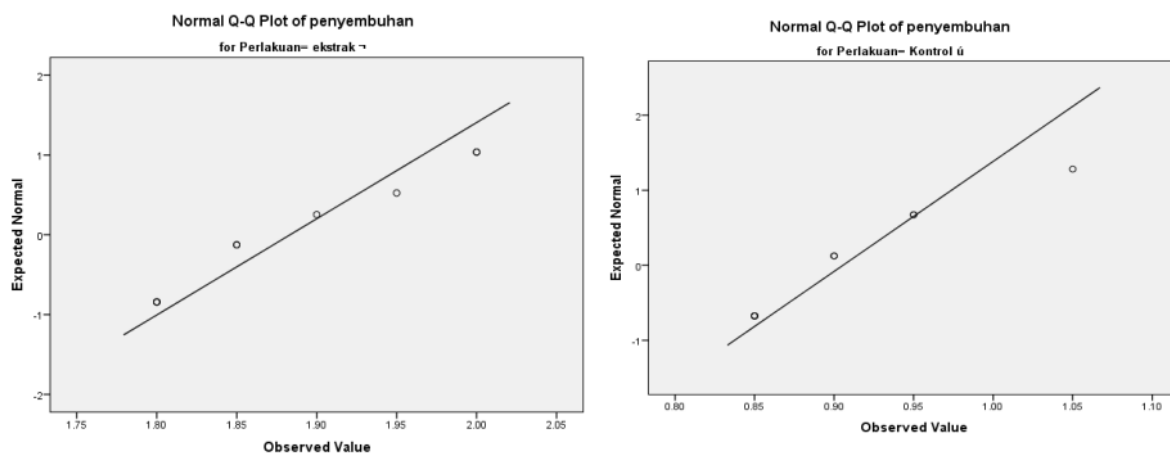
Table 2. Normality Test

Treatment	Kolmogorov-Smirnov ^a	Sig.	Information
Extract	0,212	0,200	Normal
Control	0,237	0,156	Normal

Table 2 shows a data distribution test that were used in determining the data distribution. Data Analysis that were used were similar in between group. On the extract group a Kolmogorov-Smirnov grade of 0,212 with a p-value of 0,200. A p-value score were found to be more than 0,05 therefore, in accordance with the hypothesis, that data received were distributed normally. Moreover on the control group a Kolmogorov-Smirnov score of 0,237 were found with a p-value of 0,156. P-value being more than 0,05 it can be concurred according to the hypothesis that the data distribution were normal, which is further enhanced with the following graph which follows the normal distribution :

Graph 1. Treatment Data of Extract and Control Group

According to the graph for the treatment of each group, the data distribution are well within the



normality line, this shows the data are distributed

normally.

Tabel 3. Homogeneity Test

F	df1	df2	P-value
12,253	2	24	0,000*

Table 3 shows the data homogeneity test. Obtained a statistical F value of 12,253 other than that obtained a p-value of 0,000. The p-value obtained is less than 0.0, so it can be concluded that the data is not homogeneous. ANOVA test was conducted to determine the effect of treatment on the research model carried out. The ANOVA test results are presented in the following table:

Tabel 4. ANOVA Test Result

27

Source	Type III Sum of Squares	df	Mean Square	F	P-value
Corrected Model	6,502 ^a	2	3,251	846,096	0,000*
Intercept	68,800	1	68,800	17904,675	0,000*
Treatment	6,502	2	3,251	846,096	0,000*
Error	0,092	24	0,004		
Total	75,395	27			
Corrected Total	6,595	26			

a. R Squared = .986 (Adjusted R Squared = .985)

*p-value < 0,05; Significant

46
 Table 4 shows the results of the Analysis of Variance (ANOVA) test which is based on the statistical value in the Corrected Model row obtained by 846.096. This shows that the statistical value of F is greater than the value according to the appendix $F_{tabel} = 2.621$, meaning that there is sufficient evidence to reject or be accepted. So it can be concluded that there is at least 1 influential treatment. The intercept point in the model obtained is in accordance with the suitability of the model because the Sig value obtained is smaller than 0.05, which is equal to 0.000. In addition, the F test for treatment shows that the significance value (Sig) obtained is smaller than 0.05 on the the treatment given.

35
 The number of treatments were as many as 3 treatments given in this case based on the ANOVA test provides information that there is a significant difference from each of these treatments. Therefore, it can be concluded that overall there is a significant difference between the average treatments and the length of time for wound healing. In addition, the cohort research model carried out through the ANOVA test has an accuracy level of 98.6% indicated by an R^2 value of 0.986, while the remaining 1.4% is an error or variable that cannot be explained in the ANOVA model.

45
 All of the aforementioned treatment that were done using the Saba Banana peel has a significant effect on wound healing. However, the average wound healing time of each group may differ with one another. Based on that, there needs to be an Adjunct Test (*Post- Hoc Test*)

LSD test are testing that were done in order to know each of the effect of the model treatment in the ANOVA test. LSD test make use of the average result of each treatment, therefore an average score found were as follows:

Tabel 5. Adjunct Test

Treatment	Average	Std. Error

Extract	1,883	0,021
Iodine	2,000	0,021
Control (-)	0,906	0,021
Total Treatment	1.596	0,012

Table 5 shows the statistical descriptive results for each treatment. In the extract treatment, the results showed that the average value was 1.883, while the povidone iodine variable had an average of 2,000, so the average result in the control group was 0.906. The highest average is owned by the povidone iodine variable, while the lowest average is in the Control variable. All treatments have the same standard error so that they have almost the same data distribution in the LSD test. So that the total results obtained an average total treatment of 1.596 with a standard value of data errors of 0.12. The standard data error value is much smaller than the total data. This suggests that the spread of errors can be minimized when the sample is thoroughly examined.

Based on the results of descriptive statistical analysis, it can be continued with Adjunct Test results using the LSD test. The LSD test results were obtained according to the following table: Tabel 6. Selisih Ekstrak, Iodin dengan Kontrol Negatif

Treatment		Average Difference	p-value
Control (-)	Extract	-0,9778	0,000*
	Iodine	-1,0944	0,000*

*p-value < 0,05; Significant Table 6 shows the average differences in the treatment group, namely control, Saba banana peel extract, and povidone iodine. The test results showed that the difference in the average treatment between the control treatment and the treatment of Saba banana peel extract was -0.9778. The difference value obtained showed that the average of the Saba banana peel extract group was higher than the control group, resulting in a negative average difference. In addition, obtained a p-value of 0.000 which is smaller than 0.05, so it can be concluded that the treatment group of Saba banana peel extract has an effect on the duration of wound healing. In testing the difference in the average treatment between the control group and the povidone iodine, the mean difference was -1.0944. The difference value obtained showed that the average of the Iodine group was higher than the control group, resulting in a negative average difference. The p-value obtained was 0,000 which was smaller than 0.05, so the results showed that the treatment of povidone iodine had an effect on the length of time for wound healing, to see the most influential treatment group between the Saba banana peel extract group and povidone iodine can be seen from the absolute value. the biggest difference in average, so it can be concluded that the good treatment of the povidone iodine group has the most influence on the model because it has the absolute value of the largest average difference, which is 1.094

Discussion

This study used 27 mice which were divided into 3 treatment groups. Each treatment group amounted to 9 mice, and each group was given different treatments in each group, for positive control given povidone iodine, for negative control given aquadest and for the treatment group given Kepok banana peel extract which was obtained by maceration process.

The subjects of the study were male mice, healthy, weighing 26-30 grams, aged 2-3 months and able to adapt to the laboratory environment, and those excluded as samples were mice that did not meet these criteria. The reason for selecting mice with these criteria is that these mice are more adaptive and stable during the research process²³.

The purpose of this study was to determine the impact of giving Saba banana peel extract on incision wounds in mice. The aim of giving 2 control groups was to compare the ongoing healing process and its impact on wound healing time. Saba banana peel extract has 2 main ingredients that have an impact on wound healing time, namely flavonoids and tannins^{5,7}. Flavonoids work by inhibiting cyclooxygenase and lipooxygenase so that they inhibit the occurrence of an abnormal inflammatory process¹⁷.

Flavonoids are a bioactive group commonly found in plant based foods. Besides having the ability to help the wound healing process, flavonoids also have a positive impact on general health, these impacts include the ability to prevent cardiovascular disorders. Research conducted by McCullough in 2012 cited in Kozłowska's study in 2013, showed that the level of flavonoid consumption in men and women is inversely related to the level of risk of death from cardiovascular disorders. Research by Cassidy A in 2011 also showed that consumption of flavonoids also helps in protecting the body from hypertensive conditions. The antioxidant ability of flavonoids also helps in preventing atherosclerosis, due to the ability of antioxidants to form ROS or reactive oxygen species that reduce the level of inflammation in the endothelium of blood vessels.

Flavonoids also have an impact on the nervous system and also have anti-cancer potential. Against the nervous system, flavonoids have the ability to modulate neuronal function and slow down the degeneration of the nervous system that causes conditions such as Alzheimer's, Dementia and Parkinson's, while the anti-cancer potential possessed by flavonoids is able to inhibit the proliferation and activation of cancer cells.^{25,26}

Research conducted by Ambiga S et al, showed that the application of materials containing flavonoids has an impact on the final result of the wound, which provides a more compact tissue regeneration result and is similar to normal tissue.¹⁸ The tannin content in Saba banana peel extract has bactericidal and bacteriostatic capabilities, so that the presence of Tannin in the wound healing process can minimize the obstacles to wound healing that arise due to the presence of bacteria. Tannin has antioxidant abilities that have an impact other than on wound healing abilities. It should be noted that Tannin is a polyphenol compound, just like flavonoids, so tannins also have the same systemic capabilities and effects as flavonoids, in this case they have the ability to inhibit the development of cancer cells.

Another thing that tannins have and are different from flavonoids, in the context of systemic impacts, is based on research conducted by Ghosh D et al and Mitra E in 2013 and 2014 respectively and quoted in further research from Ghosh D in 2015, showing that tannins have detoxification ability in the body for heavy metal content such as cadmium and lead.^{28,29}

The content and impact both locally on wound healing and also on systemic properties of flavonoids and tannins, shows the potential utilization of these 2 polyphenol compounds in the medical field. It should be noted that in both animal and human experiments, none has shown a negative impact from the consumption of foods containing flavonoids and tannins^{27,29}.

According to research conducted by Vipa and Chidchom cited in a study by Espinosa et al,⁴⁷ found that the Tannin content in the banana peel extract of Saba (*Musa paradaisica*) was 1130mg TAE (Tannic Acid Equivalents) / 100g of skin, this amount is higher when compared to the results of Espinosa's study. et al on 3 other banana variants, namely *Musa cavendish* (209mg / 100g) *Musa cavandanaish* and *Musa acuminata* (154.5mg / 100g) 20. Tannin works by inhibiting the process of forming substrates and enzymes from certain microorganisms so that these microorganisms experience inhibited development, tannin toxicity can damage the membranes of these microorganisms^{21,22}.

This study examined the effect of the application of Saba banana peel extract on the healing time of incision wounds in mice (*Mus musculus*) which were incised in the labial gingiva of the maxillary central incisors. The results showed that there were differences in healing time that occurred in each group. The content of flavonoids and tannins in Saba banana peel extract plays a role in wound healing time by minimizing excessive inflammation time so that the next healing phase can take place and can also minimize the number of bacteria present in the wound with the bactericidal and bacterostatic abilities of tannins^{17,21,22}. With these two components, the wound healing time can be shortened by using the Saba banana peel extract.

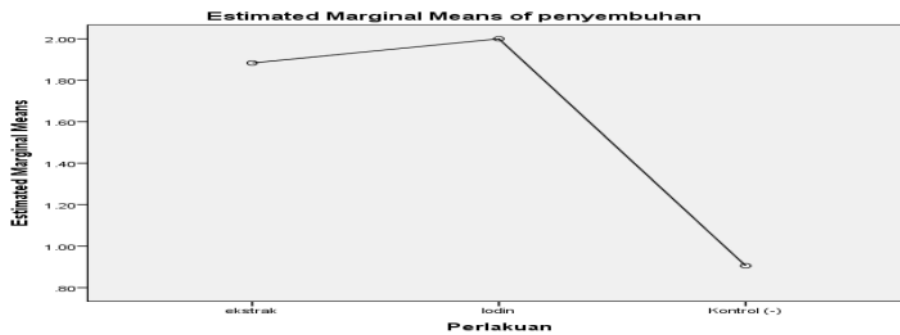
In the group given povidone iodine showed good regeneration activity, this is due to the content in the povidone iodine,³⁰ the content of the povidone polymer carrier interacts with the aqueous medium so that it releases free iodine which has antiseptic ability, with the formation of a povidone iodine complex, equilibrium will be achieved which in turn produce povidone iodine which has bactericidal and bacteriostatic abilities. Povidone Iodine has a broad range and includes a broad spectrum antiseptic so that it can be used in general^{24,25}.

⁴³ Based on table 4, the ANOVA test shows that of the three treatments given, at least one treatment has an impact on the wound healing time in mice, so what we need to pay attention to is the average healing time that occurs in the three treatments to see which treatment is used which had a greater impact on wound healing time in mice.³¹

In table 1, it can be seen that there is a significant healing using Kepok banana peel extract and also povidone iodine, this is according to research conducted by Ambiga et al. wound healing.¹⁸ The reason for the more significant healing achieved when using povidone iodine is thought to arise due to the better antimicrobial ability possessed by povidone iodine so that the wound is cleaner when compared to using

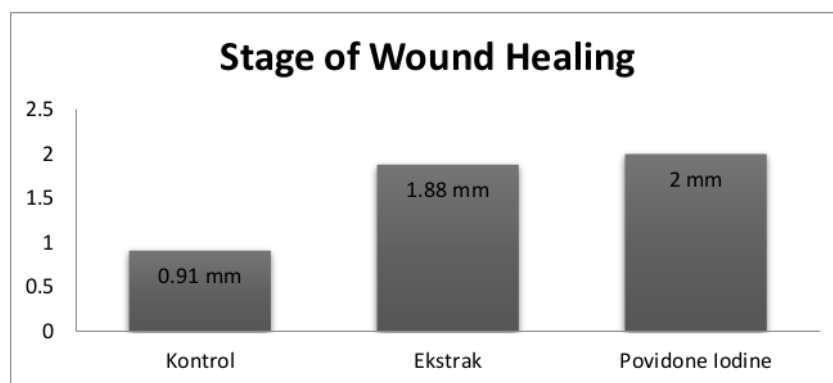
Kepok banana peel extract. included in the category of broad spectrum antibiotics so that they can have an antimicrobial effect on many types of microbes.^{24,25}

In graph 1 for the extract and positive control, it is observed that there is a consistent increase in healing time, so it can be concluded that the healing that occurs is progressive and constant during the study time, namely for 11 days. In Table 5.6, it can be concurred that the difference in healing that occurred in the negative control when compared to povidone iodine and Saba banana peel extract, which had a significant difference so that on the 11th day in each group in the negative control, none had experienced wound healing.³² The difference between negative control and povidone iodine was 1.0944 mm and with Saba banana peel extract was 0.9778 mm. The average healing found using the LSD test showed that the banana peel extract of Saba banana had an average healing rate of 1.883 mm, for povidone iodine there was 2,000 mm and in the negative control 0.906 mm. Based on the results of the study, it was found that those that had a significant impact on wound healing time and were suitable to be used as agents to help wound healing were Saba banana peel extract and povidone iodine,³³ while the order of significance for healing was povidone iodine, kepok banana peel extract and then by using sterile aquadest.³⁴



Graph 2. Wound Healing Average

The average differences of wound healing time are shown on the plot graph above, it shows that treatment using povidone iodine has a higher average in comparison with those using the Saba Banana peel.



Graph 3. Stage of Wound Healing

Graph 3 shows healing average treatment were different, on the negative control it shows an average of 0,91 mm, on the Saba banana peel extract shows a 1,88 mm average and on the povidone iodine shows a 2 mm average.

Conclusion

Based on research, Saba banana peel extract has a good ability in healing incision wounds in mice (*Mus musculus*) in terms of healing time. The ability of Saba banana peel extract is almost the same as povidone iodine because both Saba banana peel extract and povidone iodine both have bactericidal and bacteriostatic abilities to prevent bacterial infection and excessive inflammation.

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PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12
