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The Effect of Ambon Banana Stem Sap (*Musa paradisiaca* forma typica) on the Acceleration of Wound Healing Process in Mice (*Mus musculus albinus*).

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Abstract

The aim of this research is to find out the activity of banana stem sap (Musa paradisiaca forma typica) on the acceleration of wound healing process in the mice skin (Mus musculus albinus) based on gross and histopathological observations. Totally 45 heads of DDY mice ages 4-6 weeks were used in this study. The mice were divided into three groups, negative control (without treatment), positive control (Bioplacenton®) and banana stem sap. All mice were 1-1.5 cm incised on the dorsal back skin. Gross lesions were observed daily. On the 3rd, 5th, 7th, 14th and 21st days after the treatment, mice were euthanized and the skin samples were collected for further histopathological observation. The anatomical parameters were blood coagulation, dryness, attachment/narrowed of the wound and formation of the blood clot. The histopathological parameters were number of macrophages, neutrophils, lymphocytes, neo-vascularisation percentage of re-epithelization and the thickness of fibroblast. All qualitative data were statistically analyzed using Analysis of Variance (ANOVA) and continued with Duncan Multiple Range Test. Gross lesion and the fibroblast thickness were observed and descriptively analyzed as a quantitative data. The result indicated that banana stem sap could promote the wound healing process. Gross lesion observation indicated that in the banana stem sap treatment the scab formation was faster than negative control and Bioplacenton®. On macrophages, neutrophils and lymphocytes observations, the statistical analyze showed that the banana stem sap treatment was significant increase ($P<0.05$) than the negative control. The fibroblast thickness on the skin wound treated with banana stem sap was high and the formation was also faster than the negative control and Bioplacenton®. All result mentioned above indicated that Ambon banana stem sap was accelerated the wound healing process. Further study is required in order to clarify the mechanism of the sap on wound healing process as well as thir toxicity and possibility for use both in animal and human medicine.

Key words : *Banana, histopathology, mice, skin, stem sap, wound healing,*

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Introduction

Skin wound healing could be defined as a loss of integrity of the skin as a body's main barrier of outer surface. In the human and veterinary medicine, wound cases are very common such as due to surgery, traumatic, skin burn and others. Wound healing is influnced by many factors including the kind of medicine/ drugs uses. The use of drugs for wound treatment could be use in many ways and kinds; one of these kinds is the use of herbal medicine. It's already known that some plants could be use for wound treatment such as banana tree.

Skin is a main barrier for preventing the invasion of pathogenic microbes from the environment. Skin wound will facilitate the pathogenic microbes to enter the body and causing infection. The use of midicine/ drugs is aim to accelerate the wound healing process and to prevent from infection (Yahya 2005). Wound healing process could be devided in 3 phases, there are inflamation phase, proliferation phase (regeneration or fibroplasia) and re-absorbtion phase (maturation or tissue re-absorbtion). Parameters use to indentify this 3 phase are inflamatory cells (makrophages, neutrophiles and lymphocytes), neo-capilarization, re-epitelization and connective tissue (Sjamsuhidajat and De Jong, 1997; Kalangi, 2004).

Indonesia as a mega diversity country with 25.000-30.000 plant species has 6.000 species of medicinal plants (Kardono 2003). One of the potential plants to be explored as medicinal plants is banana plant. Banana tree is an indigenous plant of South East Asia including Indonesia (Munadjim 1983). This plant is growth well, easily and common found in a huge number in most South East Asian countries. People use this plant mainly for the fruit and leave; the stem is mainly use only for ruminant feed and some cultural activity, therefore the use of the stem is not yet optimally while the stem sap itself never been exploited at all. Satuhu dan Supriyadi (1995) stated that the banana stem contain serotonin, noerepinefrin, dopamine, tannin, vitamin A, vitamin B and vitamin C that are very essensially for body in the wound healing process. Serotonin could increase the function of digestive tract, decreasing the process of inflamation and stimulate the skin cell regeneration. Priosoeryanto (2003) also explained that banana stem sap contain saponin, antrakuinon dan kuinon that functioned as antibiotic and accelerated the growth of cells on the regeneration process. This stem sap also increases the blood flow and stimulates connective tissue formation on the response of wound healing process. According to Djulkarnain (1998), Ambon banana stem sap could be use for pain

reliever and facilitate the increasing of absorbtion capability of medicine in the skin therefore could be use to treated contusio, skin burn, animal bit and as anti-inflamation.

Due to many beneficial activity of the stem sap that never been explore before, we conducted the present study in order to elabotae scientifically the activity of Ambon banana stem sap on the wound healing process.

Materials and Methods

Banana Stem Sap Preparation

The banana tree was identified as Pisang Ambon (*Musa paradisiaca* forma typica) for their species and variety in the Research Center for Biology, Indonesian Institute of Science (LIPI) Bogor. The stem sap was collected directly from the stem by cuting the stem with knife aseptically.

Laboratory Animal

Totally of 45 head of mice DDY strain, 4-6 week old were used in this study. Mice were kept in the individual cage with the optimum environment and temperature (18-24^0 C). Mice were fed with a commercial feed and drinking water was given *ad libitum*. Adaptation period was done for 2 weeks.

Treatment of the Mice

Mice were distributed into 3 groups i.e. negative control, positive control (Betadine®) and treatment (stem sap) groups. Each groups were then divided into 5 sub groups according to sampling day (day 3, 5, 7, 14 and 21). On the day 12nd, the hair on the back skin were shaved and 2 days later the incision about 1-1,5 cm were done. According to the groups, mice were treated topically with Betadine® or stem sap everyday. Daily observation of the gross lesion was done twice a day (morning and afternoon). On the desired day (3, 5, 7, 14 and 21) mice were euthanized using chloroform inhalation and the skin at the incicision site and surrounding area were sampled for further histopathological process and observation..

Gross Lesion

Gross lesion examination was performed daily from day 1 to day 21 by direct observing the wound for their blood coagulation,

dryness, attachment/narrowed of the wound and formation of the blood clot.

Histopathological Findings

Histopathological observation was done by comparing the treated and un-treated groups on the parameters of the number of inflamatory cells, number of neo-capillary, percentage of re-epithelization and the density of the connective tissue. Observation for the number of inflamatory cells were for macrophages, neutrophiles and lymphocytes. Inflamatory cells and neo-vascularisation examination were performed on 15 microscopical fields with 3 replicates using a light microscope. Percentage of the re-epithelisation was done using a videomicrometer by calculating the ratio of the length area of the wound which covered by new epithelial cells with
Calculation of the re-epithelisation according to DiPietro (2001):

$$\% \text{ Re-epitelisation: } \frac{\text{Length of the wound with new epithelial cells}}{\text{Total length of the wound}} \times 100\ \%$$

Thickness of the connective tissue was examined by the intensity of the connective tissue with Masson Trichrome stained using a scoring methode as describe below.

Table 1. Scoring Lesio for the Connective Tissue

Grade	Parameters
+	The wound stil open with minimal density of connective tissue, the distance between connective tissue is loose.
++	The wound could stil open or partially closed with connective tissue density low to moderate in several areas.
+++	The wound could totally closed or minimally open with high density and compact of the connective tissue. Some loose area is still detected with formation of neo-capillaries
++++	The wound is totally closed with very high density and compact of the connective tissue

Data Analysis

The data of inflamatory cells and formation of neo-capillariy were statistically analyzed using ANOVA and followed by Duncan Multirange Test. Gross lesion and the densities of connective tissue were analyzed descriptively.

Result and Discussion

Gross Lesion

Wound healing process (daily observatio) of all groups was tabulated in Tabel 2 below.

Table 2. Gross Lesion of all Groups.

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
1	Wound still open, reddish, wet and blood cloting is present.	Wound still open, reddish, wet and blood cloting is present.	Wound still open, reddish, wet and blood cloting is present.
2	Wound still open, red to yellowish and wet	Wound still open, reddish, wet and white granule	Wound still open, reddish and wet
3	Wound still open, yellowish and minimal dry	Wound still open, minimal dry and reddish black granule	Wound still open and wet.
4	Wound still open and minimal dry	Wound still open and minimal dry	Wound still open and minimal dry
5	Wound still open, minimal dry and exudated	Wound still open but narrowed and minimal dry	Wound still open but narrowed and minimal dry
6	Wound still open, minimal dry and exudated	Wound narrowed with minimal scab formation	Wound still open and minimal dry
7	Wound still open and minimal dry	Wound narrowed with minimal scab formation	Wound still open and minimal dry
8	Wound narrowed, dry and with mininal scab fromation	Wound narrowed with minimal scab formation	Wound narrowed and minimal dry
9	Wound narrowed, dry and with minimal scab formation	Wound narrowed, dry and with moderate scab formation	Wound narrowed and minimal dry
10	Wound narrowed and dry with moderate scab formation	Wound narrowed and dry with complete scab formation	Wound narrowed, dry and with minimal scab formation
11	Wound narrowed and	Wound narrowed and	Wound narrowed,

	dry with complete scab formation	dry with complete scab formation	dry and with minimal scab formation
12	Wound narrowed and dry with complete scab formation	Wound started to closed and scab was detached	Wound narrowed and dry with complete scab formation
13	Wound started to closed and scab was detached	Wound was closed and scab trace still appeared	Wound started to closed and scab was detached
14	Wound was closed and scab trace still appeared	Wound was closed and scab trace disappeared	Wound was closed and scab trace still appeared
15	Wound was closed and scab trace still disappeared	Wound trace was disappeared	Wound was closed and scab trace still appeared
16	Wound trace was disappeared	Wound trace was disappeared	Wound was closed and scab trace was disappeared
17	Wound trace was disappeared	Wound trace was disappeared	Wound trace was disappeared
18	Wound trace was disappeared	Wound trace was disappeared and started hair growth	Wound trace was disappeared
19-21	Wound trace was disappeared and started hair growth	Wound trace was disappeared and started hair growth	Wound trace was disappeared and started hair growth

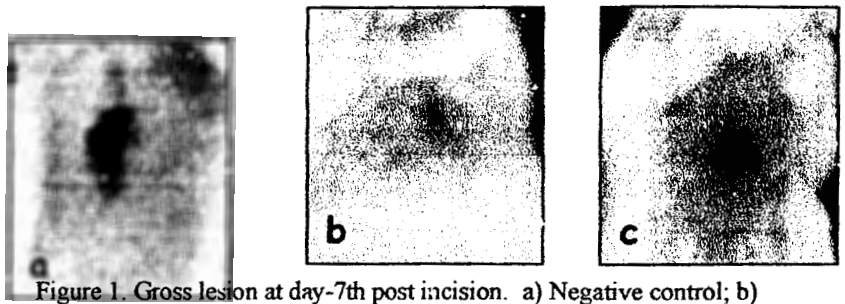

Figure 1. Gross lesion at day-7th post incision. a) Negative control; b) Banana stem sap and c). Betadine®

In the stem sap group, scab formation was started at day 7 post incision (PI), while in the Betadine® scab was formed at day 8 PI and in the negative control groups at day 10 PI. (Table 2). Scab is the manifestation of the granulation tissue, with earlier scab formation implicated that formation of granulation tissue was started earlier (Kalangi 2004). Detachment of scab and wound closed were appeared more early in the scab group, this condition implies that the skin was going back normally and the wound healing process entered the final stage. Disappering of the wound trace indicated that wound healing process was completed acheived.

Growth of the hair indicated that wounded skin were morphologically and functionally back to normal (Pinkus & Mehregan 1982). Growth of the hair was earlier detected on the stem sap group (at day 18 PI) this implies that treatment with stem sap caused wound healing process faster and better (Table 2).

Neutrophiles

Neutrophiles is the one of blood component that play an important role in the earlier response to inflamatory, phagocytosis, killing the microbes and (Lever 1986).

The appearance of neutrophiles is acted as the first leucocytes response to the acute inflamatory in order to clean up the wound from contaminant microbes by phagocytic activity (Kalangi 2004). The number of neutrophiles on day 3rd PI in the stem sap group was significantly higher compared to the Betadine® and negative control groups ($P<0.05$) as shown in Table 3. The high number of neutrophiles is indicated that the clean up and phagocytic activity was earlier occured in the stem sap group compared to other groups.

Table 3. Number of Neutrophiles

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	233.33 ± 47.93^{B}	440.00 ± 46.94^{A}	203.33 ± 30.92^{B}
5	266.67 ± 118.09^{A}	256.67 ± 8.02^{A}	171.00 ± 40.26^{A}
7	146.00 ± 65.82^{AB}	122.67 ± 45.94^{B}	232.00 ± 32.92^{A}
14	88.67 ± 14.74^{A}	60.00 ± 11.27^{A}	66.33 ± 17.67^{A}
21	94.33 ± 16.072^{A}	59.00 ± 28.58^{AB}	37.67 ± 5.1^{B}

Note: The same alphabet (superscript) indicated no significant difference (P>0.05).

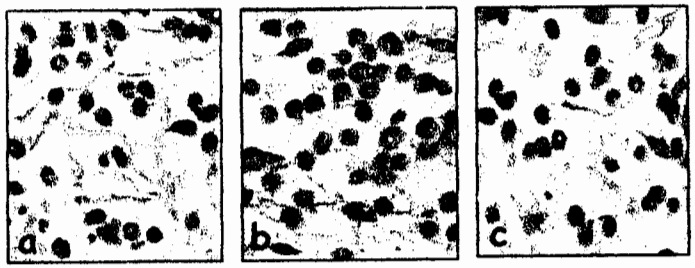

Figure 2. Number of neutrophiles day 3rd PI. a). Betadine®; b). Bananas stem sap and c).negative. Control. HE Staining. 1200X.

Kalangi (2004) stated that the present of neutrophiles in the wound is the first response of the body defense by fagocitic activity and will be decreased in line with cleaness of the wound tissue. The high number of neutrophiles in the stem sap group at day 3rd and followed by gradually decreasing up to day 21st (Table 3) indicated that the wound is clean enough from contaminant microbes therfore the requirement of neutrophiles was also decreased. The presence of neutrophiles and macrophages on the wound area is sinergistic effect in order to clean up the wound (Nadesul 2003). The function of neutrophiles as phagocytic cells for clean up the wound tissue was very optimal in the earlier stage and then was gradually replaced by macrophages in the end stage of wound healing process.

Macrophages

Macrophages is one of the bigger size of white blood cells with ability to digest the microbes, antigens and others substances which normally not circulated but present on the blood vessel associated - tissue (Yahya 2005). The function of macrophages is for fagositize and elimination (clean up) of tissue debris, killing of microbes and (Yahya 2005). The number of macrophages at day 5th PI on stem sap group was significantly high ($P<0.05$) compared to other groups (Table 4). The condition mentioned above indicated that in the stem sap groups the fagocytic activity was high compared to the other two, and this implies the faster clean up of the wounded tissue by the sap.

Table 4. Number of Macrophages.

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	212.33 ± 54.05^{A}	262.00 ± 64.13^{A}	231.33 ± 52.52^{A}
5	240.33 ± 35.22^{B}	485.00 ± 63.02^{A}	274.00 ± 15.10^{B}
7	411.67 ± 60.93^{A}	285.00 ± 8.66^{A}	385.33 ± 91.49^{A}
14	82.67 ± 23.07^{B}	139.67 ± 30.66^{A}	132.67 ± 12.90^{A}
21	76.33 ± 32.02^{A}	80.67 ± 43.98^{A}	38.67 ± 9.29^{A}

Note: The same alphabet (superscript) indicated no significant difference (P>0.05).

Biologically, macrophages released the active substances such as vasoactive mediators, chemotactic, growth factors and enzymes including proteases (Kalangi 2004). In the wound healing process, macrophages formed a granulation tissue together with neo-capillary and connective tissue. The number of macrophages on the stem sap group was higher compared to Betadine® and negative control groups in every observation day (Table 4). The high number of macrophages will produce a lot of growth factors which will stimulate the growth of new cells (cell proliferation) and faster formation of granulation tissue that affected to the acceleration of wound healing process.

Lymphocytes

In the immune systems, beside phagositoses, elimination of infectious or toxical agents is also by formation of antibody. The function of lymphocytes is as natural killer which could destroy alien substances or produce specific antibody (Guyton & Hall 1997).

Table 5. Number of Lymphocytes

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	13.67 ± 4.73^{B}	33.33 ± 9.87^{A}	14.00 ± 5.57^{B}
5	15.67 ± 1.53^{B}	27.67 ± 4.73^{A}	15.67 ± 1.15^{B}
7	9.33 ± 3.51^{A}	10.00 ± 6.08^{A}	10.33 ± 3.05^{A}
14	17.67 ± 9.50^{A}	14.00 ± 2.65^{A}	18.67 ± 14.50^{A}
21	24.00 ± 9.85^{A}	6.67 ± 4.160^{B}	14.67 ± 1.53^{AB}

Note: The same alphabet (superscript) indicated no significant difference (P>0.05).

When specific lymphocyteswas activated by antigen, lymphocytes will proliferated and produce antibody (Guyton & Hall 1997). The number of lymphocytes at day 3rd and 5th PI were significantly higher (P<0.05) compared to the Betadine® and negative control groups (Tabel 5). The high presence of lymphocytes on the stem sap group indicated that lymphocytes act as body immune system together with neutrophiles and macrophages.

In the immune system mechanism, the presence of lymphocytes could be influenced by the presence of macrophages. Pathogenic microbes or substances will be phagocytize earlier by macrophages then their antigenic substances will be released into the cytosol. These antigens will be in contact with lymphocytes which stimulate the proliferation of lymphocytes (Guyton & Hall 1997). In this study, stem sap seem could stimulated the presence of macrophages which trigerred lymphocytes to proliferate for producing antibody.

Neo-capillaries Formation

Neo-capillary formation is one of a multistage mechanism in the wound healing process which a step of connective tissue re-modelling (Vegad 1996). In this study, there was a non-significant difference (P>0.05) on all groups in the neo-capillary formation.

Table 6. Number of Neo-capilllary

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	39.67 ± 19.76^{A}	66.33 ± 26.03^{A}	65.67 ± 18.01^{A}
5	108.67 ± 30.14^{A}	163.67 ± 71.35^{A}	76.00 ± 14.53^{A}
7	155.00 ± 77.35^{A}	132.33 ± 58.20^{A}	213.67 ± 64.08^{A}
14	27.00 ± 14.53^{A}	44.33 ± 17.10^{A}	36.00 ± 1.73^{A}
21	40.33 ± 17.79^{A}	41.00 ± 29.51^{A}	20.67 ± 12.90^{A}

Note: The same alphabet (superscript) indicated no significant difference (P>0.05).

According to Vegad (1996) neo-capillary was formed in the process of granulation tissue formation which started 24 hours PI and will be in the maximum at day 5th PI in order to fullfiled the nutrient intake for cells repair.

Increasing number of neo-capillary was early appeared on the stem sap group at day 5th PI (Table 6). This phenomenon indicated that stem sap stimulate the formation of neo-capillary therefore the nutrient intake is fulfilled sufficiently for the necessity of cell proliferation and healing process. At day 7th PI the decreasing requirement of nutrient in the affected tissue will also decreasing the number of vasculary untill the oedema process was disappeared (Vegad 1996). In the stem sap group, the decreasing number of neo-capillary was detected at day 5th PI while on the other groups was noticed at day 7th PI (Table 6), this condition indicated that decreasing of oedema reaction was quickly developed in the stem sap group.

Re-epithelization

Based on Stadelman *in* Kalangi (2004), re-epithelization is one of the multistage mechanisms on wound healing process that include mobilization, migration, mitoses, and epithelial cell differentiation. These stages will re - conditioned the skin integrity. Mitoses and epithelial cell migration is functioned for re-conditioned of skin integrity. In our present study, there was no significantly difference (P>0.05) on the re-epithelization between groups (Tabel 7), this condition it seem due to no stimulation effect of stem sap on the process of re-epithelization (Figure 3.).

Table 7. Percentage of Re-epithelization

Day	Treatment		
	Betadine®	Stem Sap	Negative Control
3	0 ± 0^{A}	0 ± 0^{A}	0 ± 0^{A}
5	34 ± 15^{A}	45 ± 4^{A}	31 ± 20^{A}
7	56 ± 4.7^{A}	63 ± 32^{A}	64 ± 33^{A}
14	100 ± 0^{A}	100 ± 0^{A}	100 ± 0^{A}
21	100 ± 0^{A}	100 ± 0^{A}	100 ± 0^{A}

Note: The same alphabet (superscript) indicated no significant difference (P>0,05).

Re - epithelization is a process of repairing the skin epithelial cells to facilitate the closing of wound on healing process. If re-epithelization develops quickly, the structure formation of epidermis layer will also quickly, therefore the repairing of the skin to become normal is also stimulated (Pinkus & Mehregan 1982). At day 5th PI, the percentage of re-epithelization in the stem sap group was higher compared to others, even statisticaly there was no significantly difference (P>0.05), this implies that re-epithelization process was quickly developed in the stem sap group than the two others. At the day 14th and 21st there were a similar percentage on the re-epithelization process in all groups; this figure indicated that the body response to the repairing process of the wounded tissue has been maximum and optimally achieved.

Connective Tissue

Connective tissue is a main component on wound healing process in order to increase and repair the skin/ tissue integrity (Kalangi 2004). In the stem sap group, the score of connective tissue density was high compared to the Betadine® and negative control groups (Table 8), this figure indicated that stem sap has an effect on stimulating the development of connective tissue which influences the strength of the repaired tissue (Figure 3).

Table 8. Connective Tissue Density

Day	Treatment								
	Betadine®			Stem Sap			Negative Control		
3	+	+	+	+	++	+	+	+	+
5	+	++	++	+	++	++	++	++	+
7	++	++	++	++	++ +	++	++	++	++
14	++ +	++ +	++ +	++ ++	++ +	++ +	+++	+++	+++
21	++ ++	++ +	++ +	++ ++	++ ++	++ ++	++++	++++	+++

Note : See table 1. for the scoring level

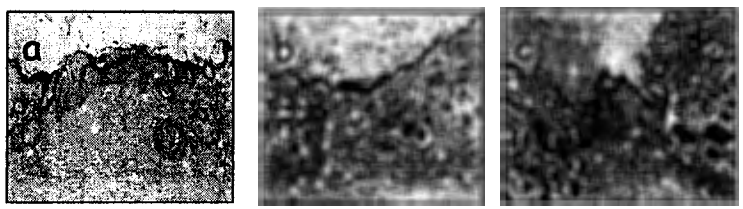

Figure 3. Microscopical appearance at day 14th PI. Negative control (a); Stem sap (b) and Betadine® (c). The number of inflamatory cells was decrease and re-epithelization was completely occured in the stem sap group. HE staining. Magnification 40X.

The main process of connective tissue growth will occured at day 7th – 14th PI and then followed by gradually increasing of tissue repairing until the normal structure of the skin/ tissue was completely achieved (Kalangi 2004). At day 14th one of the replicant on the stem sap group has achieved the maximum level (+ + + +) while the other groups achieved this level at day 21st PI (Table 8), this condition showed that the stem sap accelerated the skin/ tissue normalization.

Conclusion

1. Banana stem sap accelerated the wound healing process.
2. Banana stem sap accelerated the detachment of wound scab
3. Banana stem sap increased the number of inflamatory cells

4. Banana stem sap did not increased formation of neo-capilary and re-epithelisation
5. Banana stem sap increased the growth of connective tissue
6. Banana stem sap is seem could be use as a medicinal substance for treated the wound healing
7. Further study for clarification of the mechanism of action as well as toxicological effect of banana stem sap on the wound healing process is required.

References

Anonym. 2002. *Healing and Repair* http://medweb.bham.ac.uk/http/depts/path/Teaching/foundat/repair/healing.html. [15 Juni 2006]

.2005 *Pisang, Manfaat gizi Hingga Obat Luka* http://www.republika.co.id/suplemen/cetak_detail.asp?mid=2&id=191805&kat_id=105&kat_id1=150&kat_id2=187. [10 Juli 2006]

DiPietro LA *et al.* 2001. Wound Healing in MIP-1$\alpha^{-/-}$ and MCP-1$^{-/-}$ Mice. *American Journal of Pathology*. Burn and Shock Trauma Institute, Loyola University Medical Center. Maywood, Illinois;159:457-463. http://ajp.amjpathol.org/cgi/content/full/159/2/457/F1. [26 Mei 2006].

Fishman TD. 1995. *Phases of wound Healing* http://www.medicaledu.com/phases.htm . [10 Juli 2006]

Guyton AC, JE Hall. 1997. *Buku Ajar Fisiologi Kedokteran*. Edisi 9. Editor; Irawati Setiawan. Penerbit buku Kedokteran, Jakarta.

Kalangi SJR. 2004. *Peran Kolagen pada Persembuhan Luka.* http://www.dexa-medica.com/test/htdocs/dexamedica/article_files/kolagen.pdf. [15 Juni 2006]

Kardono LBS, N Artanti, ID Dewiyanti, T Basuki, K Padmawinata. 2003. *Selected Indonesian Medicinal Plants*: Monographs and Descriptions. PT Gramedia Widiasarana Indonesia. Jakarta.

Lever WF, SL Gundula. 1986. *Histopathology of The skin*; Histology of the Skin. Hlm. 8-36, Morphology of the Cells in the Dermal Infiltrate. Hlm. 47-55. J.B. Lippincott Company. Philadelphia.

Malole MBM, CSU Pramono. 1989. *Penggunaan Hewan Laboratorium*. Departement of Pendidikan dan Kebudayaan, Bogor.

Mayasari NLPI. 2003. *Kajian Patologi Khasiat Tanaman Lidah Buaya (Aloe vera) dalam Proses Persembuhan Luka Kulit pada Mencit (Mus musculus albinus)*. FKH IPB. Bogor.

Munadjim. 1983. *Teknologi Pengolahan Pisang*. Gramedia, Jakarta.

Nadesul H. 2003. *Biologi Sistem Kekebalan.* http://www.medicastore.com/med/detail. [15 Juni 2006]

Pinkus H, AH Mehregan. 1982. *A Guide to Dermatohistopathology 3rd Edition*; Normal Structure of Skin. Hlm 5-38, Ulcers. Hlm 193-200. Appleton Century Crofts, New York.

Priosoeryanto BP. 2003. *Penyembuh Luka di Sekitar Kita.* http://www.kompas.com/kompas-cetak/0307/24/inspirasi/451512.htm. [15 Juni 2006]

Satuhu S, A Supriyadi. 1999. *Pisang Budidaya, Pengolahan dan Prospek Pasar*. Cet. ke 10. Penebar Swadaya, Jakarta.

Soegiri J. 2006. *Tanaman Berkhasiat Indonesia*. Volume 1. IPB Press. Bogor.

Vegad JL. 1996. *A Textbook of Veterinary General Pathology: Healing and Repair*. Hlm. 134-153. Vikas Publishing House Put. Ltd, New Delini.

Yahya H. 2005. *Kastil yang Terkepung: Tubuh Manusia.* www.harunyahya.com/indo/buku/tubuh 003.htm [15 Juni 2006]