

Effect of Beluntas (*Pluchea indica* (L.) Less) Leaves Ethanol Extract to Incision Wound and Healing in Mice (*Mus musculus* L.)

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Abstract: A wound can be caused by punctures, collisions, bites or scratches of sharp object that can be avoid by mecanism of wound healing. The use of herbal medicine is an alternative choice to wound healing because of the relatively small side effects, one of which is *Pluchea indica* (L.) Less leaves ethanol extract because it contains saponin, tannin, and terpenoid as anti-inflammatory and antibacterial to accelerate the process of wound healing. The aims of this research were to find out the effectiveness of ethanol extract of beluntas leaf for duration and histological appearance process of wound healing on mice's skin. This research used 25 male mice that were divided into five different treatment groups. The treatment groups were treated with K-, K+ (povidone-iodine) and beluntas leaf ethanol extract with three concentration of 25%, 50% and 75% ointments. Incision wound was made into 1 cm length, the ointments were applied onto the wound and observed for twice a day in 14 days. Histological preparation was made to calculate epithelial thickness, lymphocytes and fibroblasts. The data were analyzed statistically using SPSS. The result of this research showed beluntas leaves ethanol extract 25% was a faster effect on the duration of wound healing which was 6.8 days. Histological observations showed that beluntas leaf ethanol extract ointment concentration of 25% had the most significant influence of the average epithelial thickness, while concentration of 75% had the most significant influence of average number of lymphocytes and fibroblast. In conclusion, the beluntas leaves extract ointment has a positive effect on the wound healing process.

1 INTRODUCTION

Wound is a condition that characterized by damage some of normal body tissues such as epithelial tissue, connective tissue, authority and skin which are often followed by nerve tissue damage and rupture of blood tissue. Wound can be caused by scratches, collisions, puncture, animal stings and other. To avoid futher damage wound healing mechanism that begins with inflammation (Abdurrahmat, 2014).

The mechanism of wound healing naturally have several phases that are inflammation phase, ploriferation phase and remodeling phase. Wound healing process requires proper care. Right eksternal condition and chemical compounds to protect wound area from contamination of microorganism and build the structure of the wound cover by itself (Handayani *et al.*, 2015).

Unritrical wound healing treatment can make inflammatory process becomes more longer, as a result the wound area becomes infected and will prolong the wound to heal (Sinaga dan Tarigan,

2012). Therefore some herbal preparations are needed as alternative choices for the process of inflammation and wound healing because have relatively smaller side effects and herbal plants are abundant in nature

Indonesia has about 40.000 medicinal plants but only about 25% have been explored by researchers and used as traditional medicine. Traditiona medicines use herbs derived from certain plants that are harmless and can be taken in an urgent situation (Marbun dan Restuasi, 2015). One of some plants that people use as traditional medicinal plants is beluntas (*Pluchea indica* (L.) Less).

Beluntas is plant of the Asteraceas family that life in hard and rocky habitats. All parts of beluntas plant are used for medicinal purposes, both roots, stems and leaves (Dalimartha, 2008). In Thailand and Java, the root is used as antipyretics, ulcers and sinusitis and leaves are used as tuberculosis durgs, thrown body odor and anti-inflammatory. In Indo-China leaves and shoots which are crushed and mixed with alcohol are used as rheumatism and scurvy (Purnobasuki, 2014; Sudirman *et al.*, 2017). Beluntas leaves as an anti-

inflammatory and astringent because of saponin and triterpenoid compound (Goyal and Agrawal, 2013).

Based on the research of Widyawati *et al.* (2014), the results of phytochemical screening of beluntas leaves using ethanol as solvents produced chemical compounds are saponin, tannin, terpenoid, flavonoid and alkaloid with have high antioxidant activity. Based on research by Puspitasari and Prayogo (2017) antioxidants can inhibit free radicals so can prevent diseases caused by radical such as liver damage, inflammation, diabetes, cancer, antiaging and wound healing.

Some researchers have reported that beluntas leaves have analgesic effects (Sibarani *et al.*, 2013), as larvicides (Muta'ali dan Purwani, 2015), as antibacterial (Rahmi *et al.*, 2015), as anti-diarrheal (Nurhalimah *et al.*, 2015) and as anti-inflammatory (Sudirman *et al.*, 2017) but it still little research on wound healing. According the phenomenon it is necessary to examine whether the ethanol extract of beluntas leaves (*Pluchea indica* (L.) Less) has an effect on wound healing in mice (*Mus musculus* L.) as information for the community so can be utilized in the future.

2 MATERIALS AND METHODS

2.1 Preparation of Sample

This research was taken in Animal Structure Laboratory, Natural Material Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, University of North Sumatera. Sample was used 25 male mice, weight 25-30 g divide into five treatment group were treated with K-, K+ (*Povidone* iodine), PI, PII and PIII which were treated by beluntas leaf extract ointment based on Febriana *et al.* (2015) have been modified with each concentration respectively 25%, 50% dan 75%. Mice were placed in a cage made from plastic material and covered by a gauze. Mice were acclimatized for 1 week and fed by ad libitum.

2.2 Beluntas Leaves Extraction

The method of making extraction is maceration. Fresh beluntas leaves were weighed and washed then dried without direct sunlight. The dried leaves were grinded to coarser powder then was macerated with ethanol 70% with ratio between powder and solvent is 1:10. Soaked for 6 hours with occasional shaking and stirring, then soaked for 18 hours again. The maserate was filtered and then repeated once again with a solvent volume half of the first process. The maserate

was evaporated with rotary evaporator at 40°C (KEMENKES RI, 2013). Beluntas leaf extract was then screened to find out secondary metabolites.

2.3 Beluntas Leaves Ethanol Extract Ointment Formulation

According to Kusumawardhani (2015), the concentration of beluntas leaves ethanol extract ointment were obtained by using the formula:

$$L = \frac{a}{b} \times 100 \% \quad (1)$$

- L : ointment concentration (%)
- a : ethanol extract of beluntas leaves (g)
- b : total weight (50 g)

Table 1: beluntas leaves ointment formulation.

Ingredients	Weight (mg)		
	25%	50%	75%
Vaseline	37,5	25	12,5
Ethanol extract of beluntas leaves	12,5	25	37,5

2.4 Treatments of Sample

Mice were anesthetized then the dorsal of mice were made shaved and a 1 cm long incision was made through the skin. Mice were treated under grouping dosing section and the ointment formulaton as described. Treatments were started from day 1 to day 14 and observed in twice a day.

2.5 Histological Assessment

The mice skin was taken on the 14th day after mice were dislocated first. Histological assessment using the paraffin method with Hematoxylin-eosin staining (Suntoro, 1983).

2.6 Observation Parameters

The parameters used were time span of wound healing for 14 days, number of lymphocyte cells, number of fibroblasts and epithelial thickness on day 14th in skin mice. Microscopical axamination used OptiLab Microscope Camera with magnifaction 100x and 400x.

2.7 Data Analysis

The data ovatined were then analyzed statistically by using SPSS software version 22.0 them using Anova test and continued with Post hoc test.

3 RESULTS

3.1 The Time Span of Wound Healing

Effect of ethanol extract of beluntas leaves for time span of wound healing for 14 days can be seen in Table 2.

Table 2: Average time span of wound healing.

Groups	Average time span \pm SD
K(-)	7.40 ^c \pm 1.14
K(+)	8.40 ^{ab} \pm 0.54
PI	6.80 ^a \pm 0.83
PII	8.20 ^c \pm 0.83
PIII	8.40 ^c \pm 1.14

Based on Table 2, it can be seen that the fastest wound healing was PI group with 6.8 days, while the longest was K+ and PIII treatments with 8.4 days. Data were tested with One Way ANOVA and obtained significant results of 0.045 ($p < 0.05$), followed by Duncan test and the results showed that the treatment group of 25% concentration of beluntas leaves was the most significant effect treatment for average time span of wound healing in mice, this maybe caused by chemical substances such as saponin and tannin which made wound healing process faster than usual.

Accordance with the research of Prasetyo *et al.* (2010) that speed of wound healing was influenced by drugs or compound that are given by stimulate the growth new cells faster. Furthermore Sudiman *et al.* (2017) said that tannin has a role in wound healing by donating hydrogen atoms to bind and neutralize free radicals so that reducing lipid autooxidation by protecting cell membranes in reducing inflammatory reaction. It is thought that this action keeps the cell membranes from being damage of bacteria to repairs during wound healing process. Parampasi dan Soemarno (2012) also said that saponin act as antibacterial that cause denaturation of proteins in bacteria so that bacterial cell membranes will be damage and finally lysis. The damage of bacterial membrane can prevent conatmination of bacterial in wound heling occurs.

Wound tretament of PII and PIII provide longer healing effect than others because it maybe the compound at that concentration did not working well so the wound healing process taken longer. The treaments also did not closed immediately due to blockage to dried up and became a scab because the concentration of extract that to high so there was a

blockage in the wound area and eventually made a new wound and heal longer.

The researchers of Dalazen *et al.* (2005) using *Vernonia scorpioides* said wound healing can be hampered because the higher concentration of extract that given, then the higher cytotoxic effect are caused. Putrianirma *et al.* (2019) said that the concentration level of the solution can also inhibit saponins to penetrate the cell membrane, also the levels of saponins that are too high can cause cell membrane permeability to increase so that the cell dies. The picture of the wound healing process in mice for 14 days can be seen in Figure 1

3.2 The Epithelial Thickness of Mice Wound

Histological observations of ephitelial thickness measured from stratum basale layer to the stratum corneum obtained avarage ephitelial thickness in five treatments can be seen in Table 3.

Table 3: Averages ephitelial thickness in mice wounds on the 14th day.

Groups	Averages of phitelial thickness \pm SD (μ m)
K(-)	95.74 ^a \pm 36.37
K(+)	101.83 ^a \pm 37.78
PI	222.55 ^c \pm 23.26
PII	180.40 ^{bc} \pm 47.56
PIII	148.74 ^b \pm 25.67

Based on Table 3, it can be seen that the highest average number of ephitelial thickness on the day 14th was in the treatment PI group with 222.55 μ m, while the lowest of ephitelial thickness was in the K- group with 95.74 μ m.

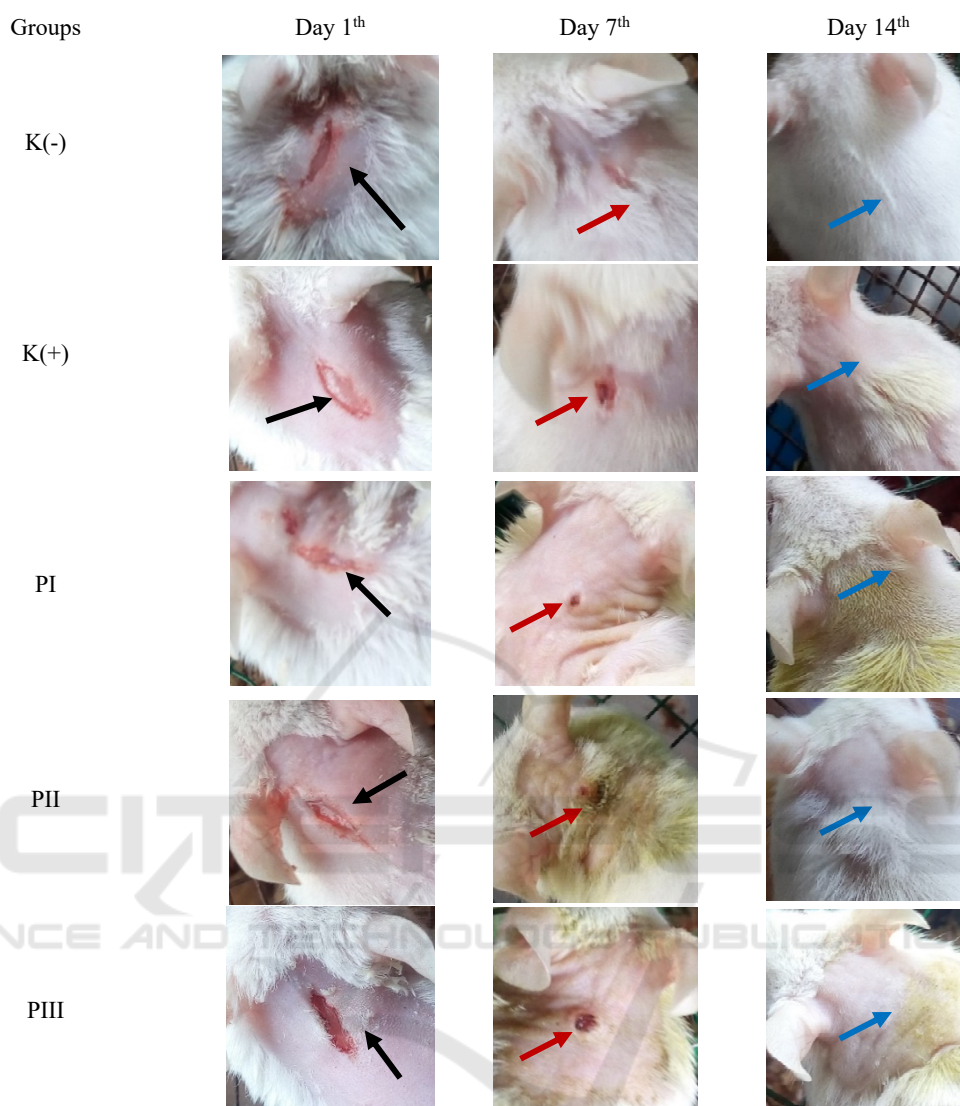


Figure 1: wound healing process of each group of treatment in mice (*Mus Musculus* L), day 1th (black arrow) wounds red and swollen, day 7th (red arrow) wounds shrink and dry and day 14th (blue arrow) wounds healed.

Data were tested with One Way ANOVA and obtained significant results of 0.00 ($p < 0.05$), followed by Duncan test and the results showed that the treatment group of 25% (PI) and concentration 50% (PII) of beluntas ethanol extract had a significant effect for epithelial thickness. High epithelial thickness in the treatment of beluntas ethanol extract ointment showed a faster reepithelization in wound healing process.

Reepithelization is an important step in wound healing, the faster the process of reepithelization, the faster the wound healed (Prasetyo *et al.*, 2010). Terpenoid compound enhance the wound healing process caused was known to have high antimicrobial

and antioxidant effects thought be liable for wound contraction and increase time span of epitheliazation of skin tissue (Wijaya *et al.*, 2014). Saponins's act in wound healing stimulating collagen type I which action in increase process of epitheliazation tissue, as an antimicrobial and accelerated epithelial cell migration (Miladiyah and Prabowo 2012).

According to Parampasi and Soemarno (2013) within 24 to 48 hours, epithelial cells move from edge of the wound along the edge of incision in dermis and precipitate compound of basale membrane along the process. These cells coalesce in the middle line of wound under the surface scap then producing an epithelial layer of wound. Proliferation of epithelial

cell caused the epidermal layer thickened. Histological preparation of epithelial thickness in mice wound 14th day can be seen in Figure 2.

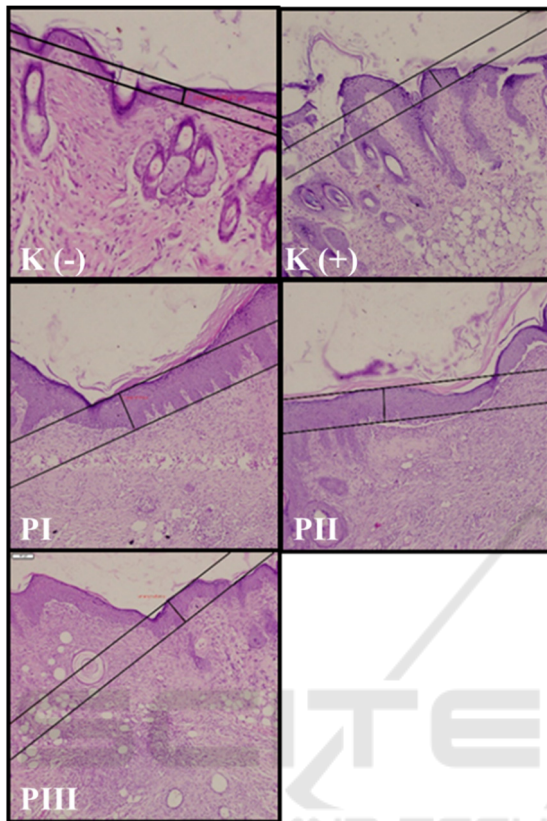


Figure 2: Histological examination of epithelial thickness wound skin in mice (*Mus musculus* L.).

3.3 The Number of Lymphocytes in Mice Wounds

Histological observations of the average number of lymphocytes on the 14th day of five treatments group can be seen in Table 4.

Table 4: Average number of lymphocyte cells.

Groups	Average number of lymphocytes ± SD
K(-)	5,64 ^a ± 3,02
K(+)	7,42 ^a ± 1,93
PI	12,24 ^b ± 2,82
PII	12,58 ^b ± 2,25
PIII	17,02 ^c ± 2,42

Based on Table 4, it can be seen that the highest average number of lymphocytes on the day 14th was PIII treatment group with 17.02, while the lowest is K- group with 5.64.

Data were tested with One Way ANOVA and obtained significant results of 0,00 ($p < 0,05$), followed by Duncan test and the results showed that the treatment group of ethanol extract beluntas leaves of 75% concentration had a significant effect for number of lymphocyte cells. The high number of lymphocyte cells indicated a wound healing process. The high number of lymphocyte cells in treatment of beluntas leaves ethanol extract maybe due to chemical compounds in the leaves of beluntas affecting the number of lymphocytes in wound area.

Wibawani *et al.* (2015) that saponin and tannin compound have antimicrobial property that can prevent and control wound infections by destroying pathogens and can reduce local inflammation and tissue damage. Izzaty *et al.* (2014) also said that role of lymphocyte in wound healing process is release lymphokines that very influential in inflammatory process by affecting the aggregation and chemotaxis of macrophages in wound area. Lymphokines is important for stimulating and activating macrophages in phagocytosis process, activated macrophages will release cytokines which will activate lymphocytes. Lymphocytes and macrophages stimulate each other to eliminate bad substances. Histological preparations of lymphocyte in the wound area 14th day can be seen in Figure 3

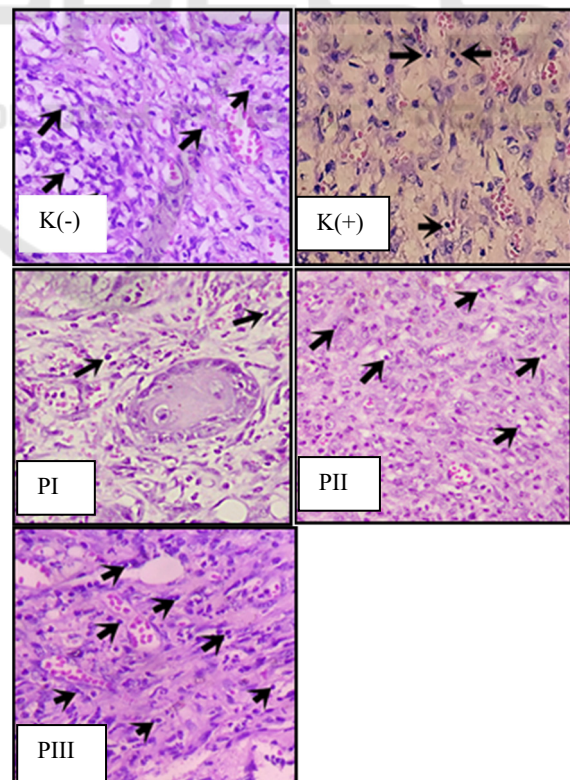


Figure 3: Histological examination of lymphocyte cells wound skin in mice (*Mus musculus* L.).

3.4 The Number of Fibroblasts in Mice Wounds

Histological observations of the average number of fibroblasts from five treatment groups can be seen in Table 5.

Table 5: Average number of fibroblasts in mice wounds 14th day.

Groups	Averages number of fibroblasts ± SD
K(-)	4,74 ^a ± 2,35
K(+)	12,32 ^b ± 3,02
PI	14,90 ^{bc} ± 5,20
PII	17,64 ^{bc} ± 2,46
PIII	20,16 ^c ± 5,34

Based on Table 5, it can be seen that the highest average number of fibroblast on the 14th day was PIII treatment group with 20.16 while the lowest was K(-) treatment group with 4.74. Data were tested by One Way ANOVA and obtained significant results of 0,00 ($p < 0,05$), followed by Duncan test and the results showed that the ethanol extract beluntas leaves (25%, 50% and 75%) had a significant effect for number of fibroblast. The large number of fibroblast in treatment of beluntas leaf extract may be caused chemical compounds in beluntas leaves such as tannins.

This is consistent with the statement of Palumpun *et al.* (2017) that tannin has a cellular mechanism activity that is eliminate free radicals, increasing the wounded connection by activating fibroblast. The tannin-containing wound stimulates the proliferation of fibroblasts and secretes collagen and proteoglycans are the main components of the extracellular matrix (ECM) and forms granulation tissue. Nurdiana *et al.* (2016) said that fibroblast does important role in the proliferation phase in wound healing process. A good wound healing process is characterized by an increase in the number of fibroblasts induced by fibroblast growth factor (FGF). The more number of fibroblasts, the more collagen will be formed, thus accelerating wound healing. Histological preparations of fibroblast cells injured area day 14 can be seen in Figure 4

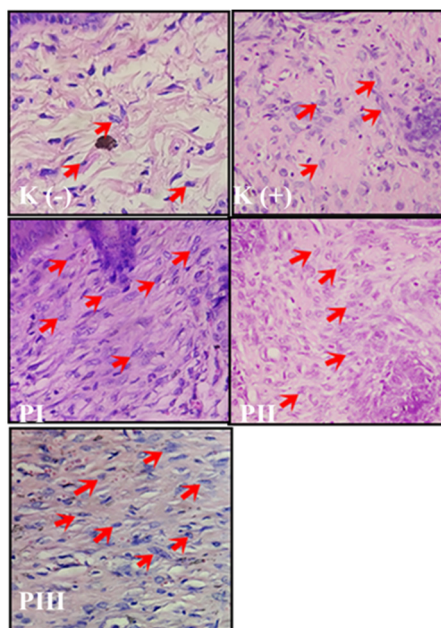


Figure 4: Histological examination number of fibroblasts wound skin in mice (*Mus musculus L.*).

4 CONCLUSION

Beluntas leaves extract ointment of 25%g gives a faster effect for time span of wound healing and average of epithelial thicknes, while concentration of 75% had the most significant influence of average number of lymphocyte and fibroblast of incision wound healing in mice (*Mus musculus L.*)

REFERENCES

- Abdurrahmat, AS., 2014. Luka, Peradangan dan Pemulihan. *Jurnal Entropi*. 9(1): 721-840.
- Dalazen, P., Uzoibian, BO., Ogbanya, KC., Nnaji, T., 2005. Effect of Topical Application of Extract Root from *Vernonia scorpooides* on Exsiccional Wounds in Mice. *Farmacogn*. 15: 82-87.
- Dalimartha, S., 2008. *Atlas Tumbuhan Obat Indonesia*. Jakarta. Pustaka Pembangunan Swadaya Nusantara.
- Febriana, MH., Amintarti, S., Putra, A., 2015. Pengaruh Ekstrak Daun Beluntas (*Pluchea indica (L.) Less*) terhadap Pertumbuhan Bakteri *Escherichia coli*. *Jurnal Wahama-Bio*. 13(1): 60-63.
- Goyal, PK, and Anggrawal, RR., 2013. A Review on Phytochemical and Biological Investigation of Plant *Pluchea*. *Indo America Journal of Pharmaceutical Research*. 3(4): 3379-3381
- Handayani, E., Siswanto, E., Pangesti, AY., 2015. Uji Aktivitas Ekstrak Etanol Gambir (*Uncaria gambir Roxb.*) terhadap Penyembuhan Luka Bakar pada Kulit

- Punggung Mencit Putih Jantan (*Mus musculus*). *Jurnal Ilmiah Manuntung*. 1(2): 134-139.
- Izzaty, A., Dewi, N., Pratiwi, DIN., 2014. Ekstrak Haruan (*Channa striata*) Secara Efektif Menurunkan Jumlah Limfosit Luka. *Dentofazial*. 13(3): 176-179.
- Kementerian Kesehatan Republik Indonesia., 2013. *Suplemen III Farmakope Herbal Indonesia Edisi I*. Kementerian Kesehatan RI. Jakarta.
- Kusumawardhani, AD., Kalsum, U., Rini, IS., 2015. Pengaruh Sediaan Salep Ekstrak Daun Sirih (*Piper betle* Linn.) terhadap Jumlah Fibroblas Luka Bakar Derajat IIA pada Tikus Putih (*Rattus novvergicus*) Galur Wistar. *Majalah Kesehatan FKUB*. 2(1): 16-28.
- Marbun, EMA., dan Restuasi M., 2015. Pengaruh Ekstrak Etanol Daun Buas-buas (*Prema pubescens* Bulme) sebagai Antiinflamasi pada Edema Kaki Tikus Putih (*Rattus novvergicus*). *J Biosains*. 1(3): 107-112.
- Miladiyah, I., and Prabowo, BZ., 2012. Ethanolic Extract of *Anredera cordifolia* (Ten.) Steenis Leaves Improve Wound Healing in Guinea pigs. *Universa Medica*. 31(3): 4-9.
- Muta'ali, R., dan Purwani, KI., 2015. Pengaruh Ekstrak Daun Beluntas (*Pluchea indica* (L.) Less) terhadap Mortalitas dan Perkembangan Larva *Spodoptera litura* F. *Jurnal Sains dan Seni*. 4(2): 55-56.
- Nurdiana, Ulya I., Putra, PRA., 2016. Pengaruh Pemberian Ekstrak Daun Melati (*Jasminum sambac* L. Ait) Terhadap Jumlah Fibroblas Kulit dalam Penyembuhan Luka Bakar Derajat II pada Tikus Putih (*Rattus novvergicus*) Galur Wistar. *Jurnal Keperawatan*. 4(1): 1-5.
- Nurhalimah, H., Wijayanti, N., Widyaningsih, TD., 2015. Efek Antidiare Ekstrak Daun Beluntas (*Pluchea indica* L.) Terhadap Mencit Jantan yang Diinduksi Bakteri *Salmonella Thypimurium*. *Jurnal Pangan dan Agroindustri*. 3(3): 1083-1094.
- Palumpun, EF., Wiraguna, AGP., Pangkahila, W., 2017. Pemberian Ekstrak Daun Sirih (*Piper betle*) Secara Topikal Meningkatkan Epidermis, Jumlah Fibroblas, dan Jumlah Kolagen dalam Proses Penyembuhan Luka pada Tikus Jantan Galur Wistar (*Rattus novvergicus*). *Jurnal I-Biomedik*. 5(1): 1-7.
- Parampasi, N., dan Soemarno T., 2013. Pengaruh Pemberian Ekstrak Daun Pepaya Etanol 70% pada Proses Penyembuhan Luka Insisi. *Majalah Patologi*. 22(1): 31-36.
- Prasetyo, BF., Wientarsih, I., Priosoeryanto, BP., 2010. Aktivitas Sediaan Gel Ekstrak Batang Pohon Pisang Ambon dalam Proses Penyembuhan Luka Sayat pada Mencit. *Jurnal Veteriner*. 11(2): 9.
- Purnobasuki, H., 2004. Potensi Mangrove Sebagai Tanaman Obat. *Jurnal Biota*. 9(2): 125-126.
- Puspitasari, AD., dan Proyogo, LS., 2017. Perbandingan Metode Ekstraksi Maserasi dan Sokletasi Terhadap Kadar Fenolik Total Ekstrak Etanol Daun Kersen (*Muntingia calabura*). *Jurnal Ilmiah Cendikia Eksakta*. 2(1): 1-2.
- Putrianiirma, R., Triakoso, N., Yunita, MN., Yudaniayanti, IS., Hamid, IS., Fikri, F., 2019. Efektifitas Ekstrak Daun Afrika (*Vernonia amygdalina*) secara Topikal untuk Reepitelisasi Penyembuhan Luka Insisi pada Tikus Putih (*Rattus novvergicus*). *Medik veteriner*. 2(1): 30-35.
- Rahmi, A., Cahyanto, T., Sujardo, T., Lestari, RI., 2015. Uji Aktivitas Antibakteri Ekstrak Daun Beluntas (*Pluchea indica* (L.) Less) terhadap *Propionibacterium acnes* Penyebab Jerawat. *Edisi Juni*. 9(1): 141-142.
- Sinaga, M., dan Tarigan, R., 2012. Penggunaan Bahan Pada Perawatan Luka. *Jurnal Keperawatan Klinis*. 3(2): 5.
- Sibarani, VR., Wowor, PM., Awaloei, H., 2013. Uji Analgesik Ekstrak Daun Beluntas (*Pluchea indica* (L.) Less) pada Mencit (*Mus musculus*). *Jurnal e-Biomedik*. 1(1): 621-622.
- Sudirman, RS., Usmar., Rahim, A., Bahar MA, 2017. Aktivitas Antiinflamasi Ekstrak Etanol Daun Beluntas (*Pluchea indica* L.) pada Model Inflamasi Terinduksi CFA. *Jurnal Farmasi Galenika*. 3(2): 191-198.
- Suntoro, SH., 1983. *Metode Pewarnaan (Histologi dan Histokimia)*. Penerbit Bhratara Karya Aksara. Jakarta.
- Wibawani, L., Endang, SW., Utami, YW., 2015. Pengaruh Pemberian Etanol Daun Melati (*Jasminum sambac* L.Ait) secara Topikal terhadap Peningkatan Kontraksi Luka Bakar Derajat II pada Tikus Putih (*Rattus novvergicus*) Galur Wistar. *Majalah kesehatan FKUB*. 2(4): 201-202.
- Widyawati, PS., Budianta, TW., Kusuma, FA., Wijaya EL, 2014. Difference of Solvent Polarity to Phytochemical Content and Antioxidant Activity of *Pluchea indica* Less leaves Extracts. *International Journal of Pharmacognosy and Phytochemical Research*. 6(4): 850-855.
- Wijaya, BA., Citraningtyas, G., Wehantous, F., 2014. Potensi Ekstrak Etanol Tangkai Daun Talas (*Colocasia esculenta* L.) Sebagai Alternatif Obat Luka pada Kulit Kelinci (*Oryctolagus cuniculus*). *Jurnal Ilmiah Farmasi*. 3(3): 2302-2493