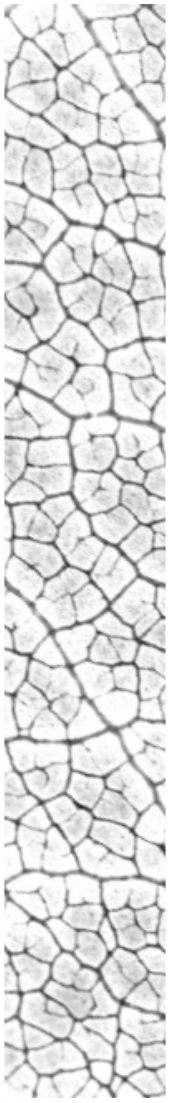

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Biological study of *Piper crocatum* leaves ethanol extract improving the skin histopathology of wistar rat wound infected by *Staphylococcus aureus*

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Abstract
The wound is a continuous damage on mucosal membrane or bone and another organ which cause a traumatic effect. This biological study aim was to investigate the effect of red betel leaves ethanol extract (*Piper crocatum*) to the skin histopathology of Wistar rat infected by *Staphylococcus aureus*. The experimental research used Randomized Post Test. Wistar male rats were divided into 5 group: P0 (control), P1 (Povidone iodine), P2 (50% of *P. crocatum* leaves extract), P3 (25% of *P. crocatum* leaves extract), and P4 (12.5% of *P. crocatum* leaves extract). The number of neutrophils, macrophage, fibroblast, and angiogenesis were analyzed by ANOVA and LSD. Collagen density was analyzed by Kruskal-Wallis test and Mann-Whitney test. This study proved that P4 group (12.5% of *P. crocatum* leaves extract) improved the skin histopathology of rat wound infected by *S. aureus*.

Keywords: angiogenesis, collagen, fibroblast, macrophage, neutrophil, red betel

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INTRODUCTION

The wound is a continuous damage on mucosal membrane or bone and another organ which cause a traumatic effect (Santoso 2011). Other research mentioned that bacterial infection slows the wound healing (Ama 2011). *Staphylococcus aureus* causes pyogenic infection and leads to sepsis (Öner et al. 2015, Sıddıka et al. 2017).

Today the medical field idea is back to nature because traditional medicine is proven safe and has no side effect like synthetic medicine. One of the traditional herbs used is *Piper crocatum* (Manoi 2007). Baroughi et al. (2013) said that *P. crocatum* extract has anti-bacterial to *Staphylococcus aureus* and *Escherichia coli*. *P. crocatum* has anti-bacterial components such as tannin, flavonoid, polyphenol, and saponin. Haryadi (2010) and Hesthisara (2011) showed that *P. crocatum* has alkaloid, polyphenols, tannin and essential oil.

There are only a few studies about the effect of *Piper crocatum* on histopathological incision infected by *S. aureus*. Thus, this study, we want to investigate the effect of *P. crocatum* extract to the skin histopathology.

MATERIALS AND METHODS

Twenty male Wistar rats divided into five random groups and each group consists of five rats. All of the groups were infected by *S. aureus* 1.5 x 10^5 CFU/0.05 ml. P0 group was negative control or no treatment group. P1 group got povidone iodine 10% as a positive control. The P2 group was treated by ethanol extract of *P. crocatum* 50%. The P3 group was treated by ethanol extract of *P. crocatum* 25%. While the P4 group was treated by ethanol extract of *P. crocatum* 12.5%. The rats were shaved on their back (3 cm x 2.5 cm) then were injected by 0.1 mL ketamine. The treatment was given 3 times a day at 06.00, 14.00 and 22.00. The ethanol extract of *P. crocatum* was given two drops using Pasteur pipette. On the tenth day, the skin histology of each rat was taken and number of neutrophils, macrophages, fibroblast, angiogenesis and collagen were gathered.

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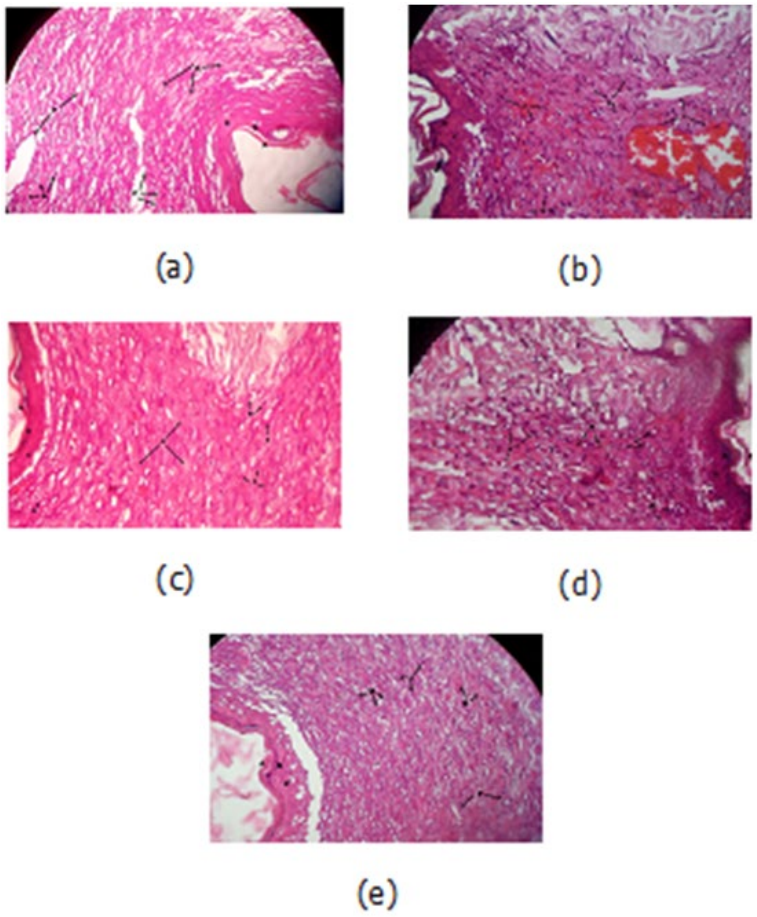

Fig. 1. The skin histopathology of P0 group (negative control) (a), P1 group (positive control) (b), P2 (50 %) group (c), P3 (25 %) group (d), P4 (12.5 %) group (e)

Table 1. Evaluation of number of neutrophils, macrophages, fibroblast, angiogenesis and collagen

Treatment	Neutrophil (x±SD)	Macrophage (x±SD)	Fibroblast (X±SD)	Angiogenesis (x±SD)	Collagen (x±SD)
P0	29.05^{a} ± 3.151	1.55^{c} ± 0.473	72.45b ± 2.941	1.40^{ab} ± 0.100	1.60^{b} ± 0.100
P1	1.10^{b} ± 0.3830	9.80^{b} ± 1.296	55.65c ± 10.585	1.30^{ab} ± 0.346	2.20^{b} ± 0.346
P2	1.05^{b} ± 0.100	10.95^{a} ± 1.660	77.40b ± 14.593	1.60^{ab} ± 0.252	3.00^{a} ± 0.252
P3	0.95^{b} ± 0.100	10.60^{b} ± 1.433	83.15b ± 13.909	2.10^{ab} ± 0.432	3.50^{a} ± 0.432
P4	1.10^{b} ± 0.4761	10.85^{b} ± 1.473	97.85a ± 4.762	2.80^{a} ± 0.661	3.80^{a} ± 0.661

Note: the different superscript showed the significant differences ($p < 0.05$)

To find the differences for each treatment the number of neutrophils, macrophages, fibroblast and angiogenesis were analyzed using ANOVA and LSD test while the collagen was analyzed using Kruskal-Wallis and Mann-Whitney.

RESULTS AND DISCUSSION

Results showed that that P4 group (12.5% of *P. crocatum* leaves extract) improved the skin histopathology of rat wound infected by *S. aureus* (**Fig. 1**).

The ANOVA test showed the significant differences among each treatment. The LSD test of neutrophil showed that P0 group significantly different ($p < 0.05$) from all treatment, while P1 group did not significantly different ($p > 0.05$) when compared to P2 (50%), P3 (25%) and P4 group (12.5%). The LSD test of fibroblast showed that the P4 group (12.5%) significantly different when compared to P2 (50%), P3 (25%) and P1 group. LSD test showed that P4 group (12.5%) significantly different when compared to P2 (50%), P3 (25%), and P1 group. The Mann-Whitney test showed that P4 (12.5%), P2 (50%) and P3 group (25%) significantly different, while P0 group did not significantly different with P1 group (**Table 1**).

The neutrophil is considered as the first leucocyte which infiltrates in wound infection. Neutrophil works by damaging and digesting the bacteria (Mathilda 2009). This process called as debridement process. Actually debridement process takes 3-5 days but in this study on the day 10, the P0 group is still in debridement process and makes the prolonged inflammation process. The previous study said that the other factor that causes

wound healing slowly is the existences of bacteria (Ama 2011).

Piper crocatum extract contains anti-bacteria such as flavonoid, alkaloid, tannin, essential oil and saponin. Flavonoid composes the complex component against extracellular protein which disturbs the integrity of the bacteria cell. Alkaloid has the ability to disturb the peptide glycan in bacteria that make the cell component doesn't develop perfectly and cause the cell death. Tannin toxicity destroys the bacteria cell membrane. Essential oil disturbs the membrane cell development process (Wurlina, 2017). Saponin is the component to develop collagen that works to heal the wound (Basaran et al. 2016). Saponin also has the ability to be antiseptic to heal opened wound (Robinson 1963).

Angiogenesis is the process of wound healing. This process has the proliferation phase. In this phase the fibroplasia and angiogenesis integrated and affected by the substance produced by platelet and macrophage. The previous study explained that the wound usually on the hypoxic and the pressure of oxygen state so the vascular do the invasion into the wound (Ama 2011).

Suratman in Basaran (2016) mentioned that saponin from *Piper scrotum* extract can increase the collagen development. The previous study said the specifical function of collagen is to create the new connective matrix tissue (Ama 2011). It gives the strength of the tissue. Fibroblast consists of mesenchyme cell whiAh has been differentiated then collagen fiber will be used for wound healing.

CONCLUSION

This study proved that P4 group (12.5% of *P. crocatum* leaves extract) improved the skin histopathology of rat wound. It can be concluded that. *P. crocatum* leaves ethanol extract improve the skin histopathology of Wistar rat wound infected by *S. aureus.*

REFERENCES

Ama F (2011) The Potency of Electric Stimulation for Skin Wound Healing in Cavia cobaya through Histopathology and Image Analysis. Thesis, Faculty of Industrial Technology, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia.

Basaran N, et al. (2016) Changing of Uric Acid Levels by Age and Sex in Patients with Diabetes Mellitus. Journal of Clinical & Experimental Investigations/Klinik ve Deneysel Arastirmalar Dergisi, 7(1). https://doi.org/10.5799/ahinjs.01.2016.01.0561

Fındık S, Görkemli H (2017) Villoglandulary papillary adenocarcinoma co-existing with high-grade squamous intraepitelial lesion; arising from an endocervical polyp. European Journal of General Medicine, 14(4): 119-21. https://doi.org/10.29333/ejgm/81745

Haryadi E (2010) Antibacterial Analysis of Piper betle and Piper crocatum Extract Against Staphylococcus aureus Growth In Vitro as Microbiological Experiment Topic. Thesis, Malang State University, Malang, Indonesia.

Hesthisara NH (2011) Antibacterial Analysis of Piper crocatum Ethanolic Extract Against Pseudomonas aeruginosa and Shigella disentriae. Undergraduate Project, Faculty of Pharmacy, Muhammdiyah University of Surakarta, Surakarta, Indonesia.

Manoi F (2007) Sirih merah sebagai tanaman multi fungsi. Warta Puslitbangbun, 13: 2.

Mathilda P (2009) Effect of Momordica charantia L Extract on Wound Healing Process in Rattus norvegicus. Undergraduate Project, Faculty of Veterinary, Airlangga University, Surabaya, Indonesia.

Öner E, Girgin S, Uslukaya Ö, Bozdağ Z, Gümüş H, Urakçı Z, et al. (2015) A survey on the factors causing delayed diagnosis and treatment of breast cancer in southeastern Anatolia. J Clin Exp Invest., 6(4): 375-81. https://doi.org/10.5799/ahinjs.01.2015.04.0552

Robinson T (1963) The Organic Constituents of Higher Plants: Their Chemistry and Interrelationships, 324 pp. Burgess Pub. Co., Minneapolis. https://doi.org/10.5962/bhl.title.6010

Santoso T (2011) Comparison of Curcuma longa Extract and Povidone Iodine in Wound Healing Process in Rattus norvegicus. Thesis, Muhammadiyah University of Yogyakarta, Yogyakarta, Indonesia.

Wurlina, Dewa KM, Mustofa I, Zakaria S, Adnyana IDPA (2017) Alkaloid Immunomodulatory Effects of Sambiloto (Andrographis paniculate L.) on the Response of Gamma interferon Gamma and T helper cell (CD4+). Advances in Natural and Applied Sciences, 11(9): 154-8.