

Effectiveness Level of Gel Combination Binahong Leaf and Turmeric Rhizome Extract on The Mature Collagen of II B Degree Burns in Rats

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ABSTRACT

The aim of this research was to evaluate the effect of administering a combination gel of binahong leaf and turmeric rhizome extracts on mature collagen of IIB degree Burns in rat. A total of 25 male rats, 3 months of age, 150-200 gram body weight were used. This research consists of five treatments. P0 were normal skin, P1 were used 1% silver sulfadiazine, P2, P3, and P4 were gel combination of binahong leaf and turmeric rhizome extracts with increase concentration of binahong leaf extract i.e. 1.25%, 2.5%, and 5%, while turmeric rhizome extract i. e 2% for each treatment. Treatments have been given topically for 14 days, twice a day, started after burn wound application. At the end of the treatment period, skin excision was carried out, then the histopathological examination was performed. Microscopic observation on the wound healing process on mature collagen density showed that P0 was not a significant difference with P3 and P4, but P0 was significant difference with P1 and P2. The better burn healing process on P3 is allegedly because of the activity of saponin, and tannin, contained in the binahong leaf extract 2.5%, and curcumin in turmeric rhizome extract 2% have been proven to be effective for topical burns therapy.

Keyword: *Binahong leaf, Collagen, Skin Burn, Turmeric rhizome, Wound Healing*

INTRODUCTION

Burns are relatively frequent and have a greater incidence in the economy and cultural marginalized countries. Every year, emergency services in the United States treat 500.000 patients with burns. Forty six percents of the cases are caused by flame and cause about 3.500 deaths/ year (Peck, 2012).

The body with burns will experience a natural healing process, if the burns occur under normal conditions; this is the response of the connective tissue. The initial phase of the wound healing process is an acute inflammatory response, which is then followed by the synthesis of collagen and other extracellular molecules in the proliferation phase. Collagen synthesis and deposit are important in the proliferation phase and the wound healing process in general (Gurtner, 2007).

Treatment of burns is very important, because the skin is the biggest organ of the body, the surface area of the skin is about 15% of the total body weight. This is also because the skin has a variety of vital functions; specifically protect viscera from external environment physical, chemical and biological substances. The skin also prevents excessive water loss from the body and plays role in thermoregulation. When the skin loses its continuity, these functions cannot work as they should. Improper treatment of wounds can inhibit the

healing process of the wound, or cause the affected area to become infected and eventually lead to chronic injury (Calais, 2014).

In several studies that have been carried out, can be seen that in rats with burns which not given topical agent or active substances or other substances that support the healing process, it will have an adverse effect because the inflammation becomes longer and also the wound healing process will be disrupted (Pongsipulung, *et al.*, 2012) and low collagen stimulation (Novriansyah, 2012).

Silver Sulfadiazine 1% (1% SSD) cream is a commonly used as a topical agent for burns therapy (Smeltzer and Bare, 2002). However, the use of Silver Sulfadiazine has negative impact towards wound healing. The negative impacts are delayed and imperfect epithelialization, formation of black scars, hypersensitivity, neutropenia, silver toxicity, ineffective against some microorganisms and thrombocytopenia (Saeidinia *et al.*, 2017).

The development of drugs or alternative agents for treating burns has been carried out for years. At present, several medicinal plants used to treat burn wounds (Meir and Nanney, 2006) such as binahong and turmeric rhizomes that can be used in healing burns. The use of binahong leaves in healing burns because the binahong leaves (*Anredera cordifolia*) are used for healing burns because it contains saponin which works as an antiseptic.

The antiseptic can stop or prevent the growth of microorganisms in the wound can avoid infection, increase the number of fibroblast cells, and stimulate collagen formation (Garmana *et al.*, 2014). In a previous study it was found that 5% of binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) could increase the collagen density of rats (*Rattus norvegicus*) with burn wounds.

Turmeric is a plant that contains essential oils. In addition, turmeric also contains curcumin. The collagen result of curcumin effect is more compact and well-aligned, bundles of collagen appeared to be thicker (Dai, *et al.*, 2009). Topically treated wounds with curcumin also showed that collagen fibres could mature earlier (Panchatcharam, 2006). In a previous study it was found that turmeric (*Curcuma longa* Linn) rhizome extract with a concentration of 2% was an effective concentration in its use as a therapy for burns.

One topical preparation is often used for treatment of burns that is gel preparation. Gel preparations have the advantage of cooling, moisturizing, easy to use, easy to penetrate the skin, and have a high-water content. This preparation is preferred because it is transparent, elastic, releases the drug well, has an attractive appearance, and does not leave an oily film on the skin thereby reducing the risk of inflammation in the skin (Prasongko *et al.*, 2020). This research aimed to knowing the effect of giving gel

combination of binahong leaf extract and turmeric rhizome extract on mature collagen density of rats (*Rattus norvegicus*) which have II B degree burns.

MATERIAL AND METHOD

This study using random sampling. The design used post-test only controlled group design is measuring results on the object of research after treatment is given. This research used a standard procedure that has been certified by the ethical board of the Faculty of Dental Medicine, Airlangga University, with certification number 307/HRECC.FODM/XI/2018.

This research was conducted in four places there are Veterinary Pharmacology Laboratory, Veterinary Pharmacy Laboratory, Veterinary Pathology Laboratory and animal experiment cage in Faculty of Veterinary Medicine Airlangga University., while it was done in October 2018 until November 2018.

The experimental animals used in this study were 25 male adult rats (*Rattus novergicus*) in healthy conditions that could be seen with agile movements, clean eyes, not attacked by fungi or other skin diseases, and had no defects, while body parts were used as samples, namely the skin. Each research object was subjected to burns with an area of 2 cm x 2 cm on the right gluteal side. On the 15th day, the skin of the

mice used as experimental animals were euthanized and placed in 10% formalin for further process in histological preparation.

The study was divided into four stages: research preparation, treatment, histopathological preparation, and histopathological assessment. At the research preparation stage, binahong leaf extract and turmeric rhizome extract were prepared using the maceration method with 96% ethanol solvent. In the first process of soaking the powdered binahong leaves and turmeric rhizome for 5 days, then filtration is carried out and the debris from the first filter is soaked again for 2 days, then filtered again and the results of the first and second filtrates are combined and re-filtered to prevent any debris, and then Each filtrate from both samples was then evaporated using a vacuum evaporator with a temperature of 60°C to obtain an almost thick extract and continued using a water bath with a temperature of 60°C to obtain a thick extract (Paju, 2013), then followed by making combination gels. At the preparation stage, the study used an adaptation to mice for 7 days.

The treatment stage includes making IIB-degree burns, which anaesthetics were administered in the form of a mixture of ketamine and xylazine intramuscularly to mice that would be given burns, before making IIB degree burns in mice. Then shave the part to be treated, namely right of gluteal rats. Alcohol 70% was applied

using cotton in the right gluteal area of the sheared rat. A second b degree or deep second-degree burn injury were made by applying the electrode that made of stainless steel to the skin purposely with 85±5°C temperature for 5 seconds to the right gluteal rat for 6 seconds to produce IIB-degree burns (Abdeldjelil *et al.*, 2017). The making of burns was carried out on all treatment groups except the treatment group T0.

After making burns, different treatments were given to each treatment group. T0 is the treatment group that was not given any treatment. The treatment group T1 was given treatment using 1% Silver Sulfadiazine (SSD) cream. Treatment group T2 was given treatment using a gel combination of binahong leaf extract and turmeric rhizome extract with a concentration of binahong extract 1.25% and a concentration of turmeric extract 2%. Treatment group T3 was given treatment using a gel combination of binahong leaf extract and turmeric rhizome extract with a concentration of binahong extract 2.5% and concentration of turmeric extract 2%. While the treatment group T4 was given treatment using a gel combination of binahong leaf extract and turmeric rhizome extract with a concentration of binahong extract 5% and concentration of turmeric extract 2%. The treatment was carried out for 15 days, and twice a day was given.

In the next stage, euthanasia was carried out on the fifteenth day by

administering anesthesia in the form of a mixture of ketamine and xylazine via intramuscular first, followed by cervical dislocation. The cervical dislocation method was chosen because the experimental animals used weighed under 200 grams (The University of Texas, 2020), then samples from the skin of rats measuring 2 x 2 cm were taken. The skin sample taken has been prepared with HE dye.

In the last stage, a histopathological assessment was carried out, where the histopathological assessment was based

on calculating the percentage of collagen density and collagen maturity (Table 1.) according to Shakya *et al.*, (2016) which has been modified on the score and percentage of mature Collagen Density. The results of the sample assessment which were observed histopathological were analysed by non-parametric Kruskal-Wallis, followed by Mann-Whitney U different test using the Statistics Product Service Solution (SPSS) version 20 program for Windows.

Table 1. Mature Collagen Density Assesment

Score	Status
1	Mature collagen with a density > 50%
2	Mature collagen with a density 31% - 50%
3	Mature collagen with a density 10% - 30%
4	Mature collagen with a density < 10%
5	Immature collagen with a density > 50%
6	Immature collagen with a density 31 - 50%
7	Immature collagen with a density 10 -30%
8	Immature collagen with a density < 10%

Source: Shakya *et al.*, 2016 with modification

RESULTS

The observation of macroscopic changes that occur in second-degree burns was carried out on the fifteenth day of the study conducted in the treatment groups T1, T2, T3 and T4 which can be seen in Figure 1.

On the fifteenth day can be seen in figure 1. that all treatment groups have undergone complete scab peeling, but it can be seen that in the treatment group T1 and T2 scars still appear reddish the burns scars. In contrast to the treatment groups T3 and T4, which can be seen at the site of the occurrence of burns, it only appears as little reddish or even no

reddish. In addition, it can be seen the results of the histopathology features of the skin taken on the fifteenth day showed that in each group of

experiments there was a difference in density of collagen fibres, as seen in Figure 2.

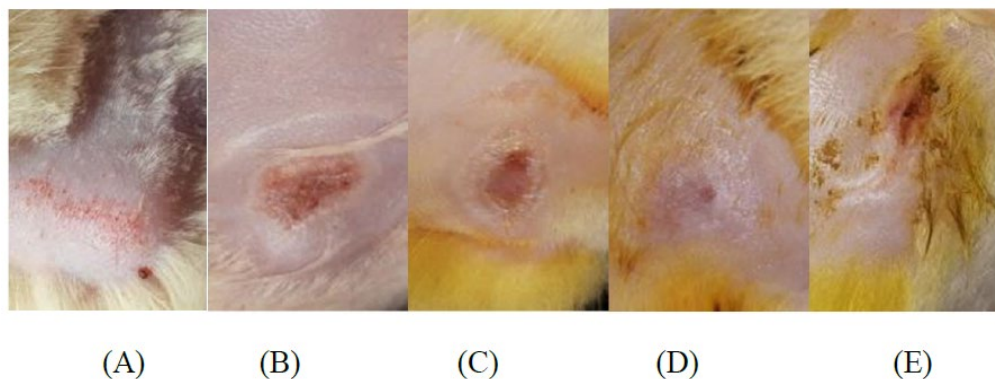


Figure 1. Macroscopic observations on the fifteenth day of research: (A) T0 (control (-)); (B) T1 (Control (+)); (C) T2 (gel with concentration 1.25% of binahong extract and 2% of turmeric rhizome); (D) T3 (gel with concentration 2.5% of binahong extract and 2% of turmeric rhizome); (E) T4 (gel with concentration 5% of binahong extract and 2% of turmeric rhizome)

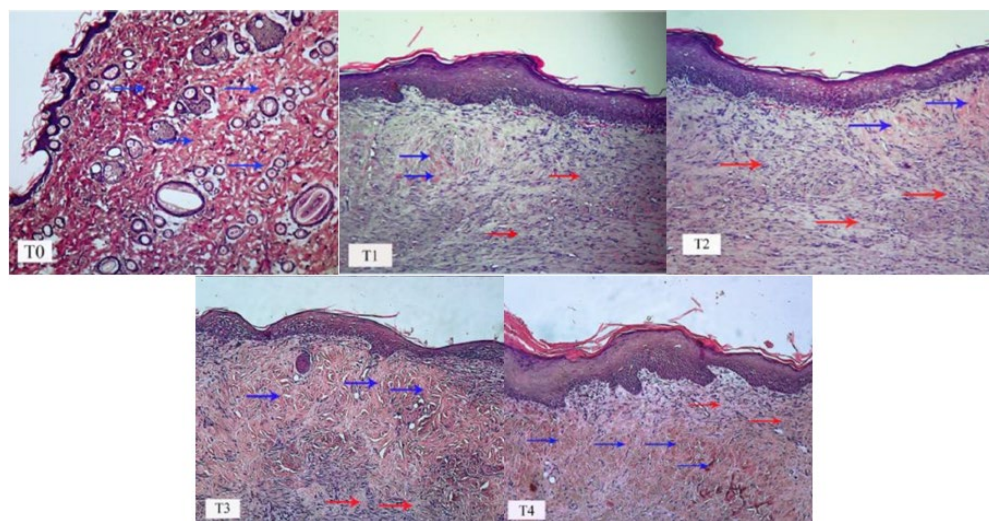


Figure 2. Histopathological Figures of Mature collagen density (→) (Type I collagen or mature collagen shows a dark pink or red color with dense spacing and density) and Immature collagen (→) (Collagen type III or immature collagen shows a rather pale pink color with a tenuous distance and density) (HE 100x) in each treatment. T0 has a normal histopathology of the skin. T1 and T2 have dense collagen fibres, although the mature collagen fibres are at 10% , it is not clearly seen. T3 and T4 both have more than 50% mature collagen fibres which can be seen from the eosinophilic coloring.

In figure 2 it can be seen that in the treatment group P0 there is a normal histopathology features of the skin which can be seen also there are many images of mature collagen fibres. In treatment group T1 and treatment group T2, there was a solid collagen fibre but mature collagen fibres that appeared were less than 10%. Whereas in the treatment groups T3 and T4 it

was seen that the density of collagen fibres was not as dense as the treatment group T1 and T2 but had more than 50% mature collagen which was more than the treatment group T1 and T2.

Based on the histopathological picture in Figure 2, the median collagen density score was obtained as shown in Table 2.

Table 2. Median Density of Collagen of Rat Skin on Each Treatment

Treatment	Median	Mode
T0	1 ^a	1
T1	4 ^b	4
T2	4 ^b	4 and 2
T3	1 ^a	1
T4	1 ^a	1

Note: Different superscripts in the same column show significant differences ($p < 0.05$).

According to Table 2 the median score of collagen density in the treatment group T0 is 1 which has a significant difference to the median treatment group T1 and T2 which has a value of 4, but does not have a significant difference with the median treatment group T3 and T4 which has a median value 1. The treatment group T1 and T2 had a significant difference in median diameter with the treatment groups T3 and T4, while the treatment group T1 did not have a median collagen score which differed significantly from the treatment group T2. The treatment groups T3 and T4 did not have a median value of collagen

density scores that differed significantly from each other. From the data in Table 2 it can also be seen that those that show the best density score are treatment groups T3 and T4.

Scores that often appear or mode in treatment groups T0, T3 and T4 are scores 1. Unlike the T1 treatment group which has a score of 4 as a mode. While the treatment group T2 has more than one mode value, namely 2 and 4, but the smallest mode value is 2.

In addition, the results of the nonparametric statistical test of Kruskal-Wallis collagen density score were $P = 0.002$ which showed a significant difference in all treatment

groups ($p < 0.05$). The differences in each group were followed by the Mann-Whitney U difference test which showed that T0 was not significantly different ($p > 0.05$) with T3 and T4, but differed significantly ($p < 0.05$) with T1 and T2, whereas T1 was not significantly different ($p < 0.05$) with T2, but was significantly different ($p > 0.05$) with T4 and T3, as can be seen in Table 2.

DISCUSSION

The results of this study indicate that the gel combined with binahong leaf extract and turmeric rhizome extract can increase the density of immature collagen and accelerate the maturity of collagen so that it can accelerate the healing process of burns. Gel combination with a concentration of binahong leaf extract 2.5% and concentration of turmeric rhizome extract 2% were said to be the best dosage because it shows the same results as normal collagen structure although it contains smaller doses than the gel combination with a concentration of binahong leaf extract 5% and concentration of turmeric rhizome extract 2%. This assessment is based on the research of Shakya, *et al.*, (2016) which has been modified where in its research the best results on the formation of collagen density show the same results as the structure of collagen in normal skin.

The difference in density and maturity of collagen in this study was also seen significantly between rats with healthy and normal skin with rats subjected to burns and then treated use 1% silver sulfadiazine. Collagen density and maturity in rats with burns treated use 1% sulfadiazine silver did not get maximum results. This is because 1% of SSD creams can damage keratinocytes which play a role in the process of re-epithelization of wounds, and cause damage to fibroblasts that disrupt the maturation process of the collagen matrix (Lee and Moon, 2003). Other study conducted by Theresia (2019) showed that rat given treatment using 1% silver sulfadiazine (SSD) cream showed a moderate value of fibroblast which value was lower than the fibroblast value in mice affected by burns that were given treatment with gel a combination of binahong leaf extract 5% and turmeric rhizome extract 2%.

Collagen density and maturity in rats with burns treated use 1% SSD cream and rats with burns treated using gel combination of binahong leaf extract 1.25% and turmeric rhizome extract 2% showed no significant differences and could be said to have poor histopathology status. It is considered that 1% SSD cream and gel combination of binahong leaf extract 1.25% and turmeric rhizome extract 2% have the same ability in the healing process of burns. Rats with burns treated using gel combination of binahong leaf extract

1.25% and turmeric rhizome extract 2% showed less optimal results, possibly this could be caused by the concentration of binahong leaf extract contained in the gel too little, so the content of the compound in the extract was also small. The concentration of plant extracts that are too low will only contain a small amount of saponins. Thus the healing process of burns that occur is also not optimal due to lack of saponin in stimulating collagen (Paramita, 2016).

In this study also found that the density and maturity of collagen in rats with healthy skin didn't show a significant difference with T3 (rats with burns were given treatment using gel combination of binahong leaf extract 2.5% and turmeric rhizome extract 2%) and T4 (rats with burns were given treatment gel combination of binahong leaf extract 5% and turmeric rhizome extract 2%). the used of gel combination binahong leaf extract and turmeric rhizome extract can increase the formation, maturation and deposition of collagen in the process of healing burns, this is related to the content in binahong leaves and turmeric rhizome. This is because binahong leaves contain bioactive compounds such as flavonoid, saponin, and tannin. The flavonoid in the leaves of binahong has an antiinflammation effect, while saponin works as an antiseptic that can terminate or prevent the growth of microorganism in the wound to avoid an infection, increase the number of

fibroblast cells, and stimulate the formation of collagen (Garmana, *et al*, 2016).

Saponins can inhibit the action of the cyclooxygenase enzyme by catalysing the reaction of arachidonic acid into endoperoxides compounds. In this case cyclooxygenase plays a role in the process of forming prostaglandins. Because the action of cyclooxygenase is inhibited, prostaglandins formation decreases, which results in a shorter inflammatory reaction and accelerates the healing process by increasing collagenization (Rizqah, 2007). Suppression of prostaglandins as inflammatory mediators can lead to reduced pain and swelling, reduce the occurrence of vasodilation of blood vessels in the local bloodstream, so that the migration of M1 (pro-inflammatory) macrophages will decrease which will then be replaced by fibroblasts. Fewer macrophages on days 7 and 14 indicated that immune cells were successful in removing irritants and cellular debris so that inflammation did not continue (Dwita *et al.*, 2020).

Binahong leaves also contain active compounds called oleanolic acid, which works as an anti-inflammatory by inhibiting COX-2 in the absence of prostaglandin formation which serves as a chemoattractant for inflammatory cell migration. Furthermore oleanolic acid will also inhibit 5-lipoxygenase thereby inhibiting the formation of leukotriene from arachidonic acid, which is a potent chemotaxis for

inflammatory cells. As a result, the inflammatory reaction becomes shorter and the formation of collagen increases so that wound healing occurs perfectly (Hapsari, 2017).

Turmeric rhizome contains various chemical components that can be used in various diseases, but the most common chemical content is curcumin. The giving of curcumin resulting collagen is more compact and well-aligned and the bundles of the collagen appeared to be thicker (Dai, *et al.*, 2009). The use of the same dose or concentration of turmeric rhizome in each gel preparation, because in a previous study conducted by Mehrabani *et al* (2014) showed that the use of turmeric extract at a dose or concentration of 2% for burns in rats showed the best results, especially increasing maturation and improve collagen deposition

The curcumin content in turmeric rhizome can increased the levels of hexosamine and uranic acid. Hexosamine and uranic acid are matrix molecules, which act as ground substratum for the synthesis of new extracellular matrix (Pillai,*et al.*, 2010; Ali *et al.*, 2022). Still uranic acid in the wound attracts fibroblasts and stimulates collagen synthesis by providing more fluid which facilitates greater cell mobility, early remodelling and assists the wounds to heal faster without scar formation (Panchatcharam, 2006). Curcumin also can accelerate collagen maturation in

the wound-healing process (Sidhu, *et al.*, 1998; Sidhu, *et al.*, 1999). Any increase in collagen synthesis is leading to a raise in newly formed collagen which is associated with an expansion in aldehyde that leads to greater potential for cross-link formation (Panchatcharam, 2006). The more cross-links in intramolecular and intermolecular of collagen, the higher the wound healing strength will be. Therefore, collagen forms tight cross-links to other collagen with protein molecules, increasing the healing wound's tensile strength (Qing, 2017).

The ability of T3 (rats with burns treated with gel combination of binahong leaf extract 2.5% and turmeric rhizome extract 2%) and T4 (rats with burns treated with gel combination of binahong leaf extract 5% and turmeric rhizome extract 2%) in accelerating the healing process of burns has the best status in this study. However, the T3 treatment group (rats with burns were given treatment using a gel combination of binahong leaf extract 2.5% and turmeric rhizome extract 2%) were able to show a good effect on mature collagen density in existing burns. Even with a smaller concentration than T4, it has the right composition so that it can show optimal results. This shows that there is a synergistic interaction between binahong leaf extract and turmeric rhizome extract. The effect of synergism is the effect that occurs when two or more types of drugs were given either

simultaneously or almost simultaneously in one drug formula or with a separate formula can result with an increase in the effect of the drug caused (Kurnijasanti, *et al.*, 2017). This shows that the dosage of binahong leaf extract which is smaller than the effective dose of binahong leaf extract (5%) when giving binahong leaf extract alone, can show better results with the addition of turmeric extract.

In addition, this result can be caused by factors that can accelerate the healing process of burns. Oxygenation is one of the factors that are also very important in the healing process of burns. This is because oxygenation can induce angiogenesis, increase keratinocyte differentiation, migrate and re-epithelise, increase fibroblast proliferation and collagen synthesis, promote wound contraction and can prevent infection (Guo and DiPietro, 2010). In addition, another factor is moisture. Low humidity will cause the oxygen pressure in the wound tissue to decrease, thus affecting the function of neutrophils, macrophages and fibroblasts and causing the collagen synthesis process to be inhibited (Novriansyah, 2008).

CONCLUSION

The conclusion of this study that the administration of gel combination of binahong leaf extract 2.5% and turmeric rhizome extract 2% can increase the

density and maturity of collagen in rats (*Rattus norvegicus*) which have II b degree burns.

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