

# Binahong.pdf

*by* Totong Kamaluddin1

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


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# Efficacy of Topical Binahong Leaf Ethanolic Extract Administration on Serum TGF- $\beta$ 1 in Wistar Rats with *Staphylococcus aureus*-infected Wounds

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**Keywords:** Anredera Cordifolia, Wound Infection, Transforming Growth Factor Beta, Wound Healing, In Vivo


**Abstract:** Binahong (*Anredera cordifolia* (Ten.) Steenis) is a medicinal plant traditionally used as a topical wound treatment. Saponin content in Binahong leaf extract has been thought to increase TGF- $\beta$  secretion, which plays an important role in accelerating wound healing. This study's aim was to assess the efficacy of topical Binahong leaf ethanolic extract administration on serum TGF- $\beta$ 1 in infected wounds. An experimental study, in vivo, was conducted in the Biotechnology Laboratory and Animal House, Faculty of Medicine, Universitas Sriwijaya, Palembang, from July to September 2020. Thirty male Wistar rats aged 10-12 weeks with excisional wounds were infected with *Staphylococcus aureus* ATCC 25923. The rats were divided into five groups and received three concentrations of Binahong leaf extracts (2.5%, 5%, and 10%), salve base, and povidone iodine 10% topically twice daily for 14 days. Serum was obtained before treatment and after day 14 of treatment. Wound area was also recorded. After 14 days of topical administration of Binahong leaf extract on rats with *Staphylococcus aureus*-infected wounds, a decrease in wound size was most significantly observed in rats receiving 10% Binahong leaf extract ( $p = 0.02$ ), but no significant serum TGF- $\beta$ 1 increase was observed in all treatment groups.


## 1 INTRODUCTION

The presence of wounds poses a risk of colonization and infection by pathogenic bacteria on the wounded site, which might spread and cause systemic infection if it is not adequately managed (Negut, Grumezescu, and Grumezescu 2018). One of the most common causative pathogens in wound and skin infections is *Staphylococcus aureus* (Roy et al. 2018). *S. aureus* disrupts normal wound healing process through the release of extracellular adherence protein (Eap). Eap release prolongs inflammation and prevents angiogenesis, especially in the proliferative phase of wound healing (Pereira-franchi et al. 2017; Wong, Manikam, and Muniandy 2015). In Indonesia, a multicenter study reported *S. aureus* isolation from 45.3% patients with wound infections and SSTI (Santosaningsih et al. 2018).

The wound healing process involves a complex interaction between the skin's cellular components, such as keratinocytes, fibroblasts, vascular endothelia, immune system cells, and extracellular matrix (Martin and Nunan 2015). These cells interact by secreting various mediators and growth factors, one of which is transforming growth factor-beta (TGF- $\beta$ ). TGF- $\beta$  is a pleiotropic growth factor secreted by platelets, fibroblasts, and proinflammatory cells (Lichtman, Otero-vinas, and Falanga 2016; Sutrisno et al. 2018). TGF- $\beta$  has an extensive role in wound healing, such as stimulating collagen synthesis, angiogenesis, and keratinocyte migration (Lichtman, Otero-vinas, and Falanga 2016; Tejiram et al. 2016). TGF- $\beta$  may also induce epithelial-mesenchymal transition, which is an important morphogenetic event in the formation of scar tissue and regeneration (Martin and Nunan 2015; Qi et al. 2018). TGF- $\beta$  has three known isoforms,

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TGF- $\beta$ 1 to TGF- $\beta$ 3, in which TGF- $\beta$ 1 is the most abundant (Wang et al. 2017).

Recently, while topical therapy remains an important modality in wound management, it has become a concern that most available topical therapies do not give additional benefits in speeding up the wound healing process (Powers et al. 2019). In case of infected wounds, rising tolerance and resistance towards common topical antibiotics and antiseptic agents also impose a challenge in choosing the appropriate treatment (Hardy et al. 2018).

Natural products and their active compounds are starting to be considered in the development of novel products for wound management (Istyastono and Yuliani 2016). *Anredera cordifolia* (Ten.) Steenis (Binahong) is a plant from the *Basellaceae* family, which has long been used in traditional medicine around the world (Astuti et al. 2011; Leliqia, Sukandar, and Fidrianny 2017). In Indonesia, Binahong has been traditionally used for various diseases, including in the treatment of wounds and bacterial infections (Astuti et al. 2011; Sukandar and Kurniati 2014).

A number of studies have been conducted on the efficacy of *A. cordifolia* extract in wounds, and so far there have been no data yet on the *in vivo* efficacy of *A. cordifolia* extract on TGF- $\beta$ 1 concentrations in infected wounds. This study aimed to determine the efficacy of *A. cordifolia* ethanolic extract on increasing serum TGF- $\beta$ 1 in rats with *Staphylococcus aureus*-infected wounds.

## 2 MATERIALS AND METHODS

An experimental study, *in vivo*, was conducted at the Biotechnology Laboratory and Animal House, Faculty of Medicine, Universitas Sriwijaya, Palembang, in July to September 2020. The study population was male Wistar rats. There were 30 male Wistar rats aged 10-12 weeks, weighing 150-200 grams which fulfilled the inclusion criteria.

Rats showing signs of infection in the first 24 hours after *S. aureus* inoculation were included in this study. Ethical clearance had been approved by Health Research Ethics Committee, Faculty of Medicine, Universitas Sriwijaya, prior to the commencement of the study (Certificate No. 024/kepkrsmhfkunsri/2020).

### 2.1 Extract Preparation

Four hundred grams of dried Binahong leaves obtained from Karangpandan, Tawangmangu, Central Java (elevation of 800 m above sea level), was extracted by maceration with 96% ethanol which is in accordance with the Indonesian Herbal Pharmacopoeia and concentrated through rotary evaporation (Kementerian Kesehatan Republik Indonesia 2009). The obtained concentrated extract was then formulated into salve with vaseline album and adeps lanae base. Three concentrations of salves were formulated, each containing 2.5%, 5%, and 10% Binahong leaf ethanolic extract, respectively. Salve base was used as negative control and povidone iodine 10% (Betadine®, PT Mahakam Beta Farma, Jakarta, Indonesia, Batch No. GB20045) was used as positive control.

### 2.2 In Vivo Efficacy Test

Rats were anesthetized by using ketamine, and their dorsal skin was depilated with scissors and depilatory cream before wounding. A 2 cm<sup>2</sup> circular excision wound was made with scalpels and surgical scissors, followed by inoculation with *Staphylococcus aureus* ATCC 25923 suspension containing  $2 \times 10^7$  cfu and a 24-hour incubation period. The rats were then divided into 5 treatment groups, each receiving salve base (negative control), three concentrations of Binahong leaf ethanolic extract salves, and povidone iodine 10% (positive control). All groups received treatments twice daily for 14 days.

Wounds were photographed on days 4, 7, 10, and 14 of treatment, and wound area was measured with image processing software. Serum samples were obtained during the 24-hour incubation period before treatment started and on the 14<sup>th</sup> day of treatment. TGF- $\beta$ 1 assay was performed by ELISA (MyBioSource, San Diego, CA, USA), following protocols specified by the manufacturer.

### 2.3 Statistical Analysis

Homogeneity and normality of data was assessed prior to further analysis. Efficacy of each treatment was assessed by using paired T test. Efficacy comparison between treatment groups and controls was performed by using unpaired T test, and significance test was performed with Post Hoc test. Significance is assumed at  $p < 0.05$ .

Table 1: Efficacy of topical Binahong leaf ethanolic extract on decreasing wound area.

Treatment Group	Wound area (cm <sup>2</sup> )		p
	Pre-treatment Mean	Post-treatment Mean	
Salve base	2.403	0.356	0.001
Binahong leaf ethanolic extract	2.368	0.225	0.001
2.5%	1.427	0.259	0.001
5%	2.069	0.198	0.001
10%	2.208	0.302	0.001
Povidone iodine 10%			

Paired T test, p = 0.05

### 3 RESULTS

Wound area was significantly decreased in all treatment groups (Table 1).

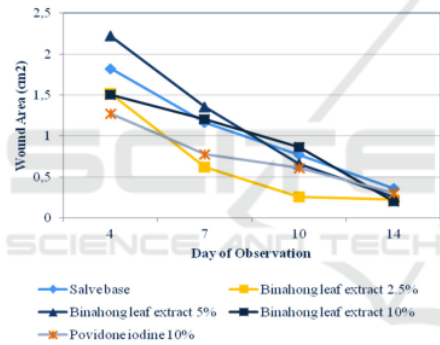


Figure 1: Wound area contraction in all treatment groups during the course of the experiment.

The most significant decrease in wound area was found in treatment groups receiving 10% Binahong leaf extract, where a significant difference in efficacy was found in comparison to negative control group (p = 0.02), but there was no significant efficacy difference in comparison to povidone iodine 10% (Table 2).

After 14 days of topical Binahong leaf ethanolic extract administration, there was no significant increase in serum TGF-β1 concentration in all treatment groups (Table 3).

### 4 DISCUSSION

This study aimed to assess the efficacy of topical Binahong leaf ethanolic extract administration on serum TGF-β1 in infected wounds, while its efficacy in decreasing wound area was also examined for baseline data. Topical administration of Binahong leaf ethanolic extract did not increase TGF-β1 levels, but effectively decreased wound area. Treatment with 10% Binahong leaf ethanolic extract was shown to be most efficacious in decreasing wound area.

Table 2: Efficacy comparison between different doses of topical Binahong leaf ethanolic extract and controls on decreasing wound area after 14 days of administration.

Comparison Group	Treatment Group	p
Salve base (negative control)	2.5% Binahong leaf extract	0.129
	5% Binahong leaf extract	0.109
	10% Binahong leaf extract	0.022
	Povidone iodine 10%	0.427
Povidone iodine 10% (positive control)	Salve base	0.427
	2.5% Binahong leaf extract	0.323
	5% Binahong leaf extract	0.360
	10% Binahong leaf extract	0.063

Unpaired T test, p = 0.05

Table 3: Efficacy of topical Binahong leaf ethanolic extract on decreasing wound area.

Treatment Group	Serum TGF-β1 (pg/ml)		p
	Pre-treatment Mean	Post-treatment Mean	
Salve base	1,036.736	1,076.681	0.128
Binahong leaf ethanolic extract	1,034.923	952.615	0.225
2.5%	1,018.898	998.705	0.753
5%	1,065.949	969.090	0.463
10%	1,072.551	1,006.461	0.249
Povidone iodine 10%			

Paired T test, p = 0.05

Presently, studies conducted on the efficacy of Binahong leaf extract in wound healing have reported various findings. A study by Paju, Yamlean and Kojong (2013) in rabbits with incisional wounds reported the efficacy of 10% Binahong leaf ethanolic extract in wound healing, which is similar to our findings (Paju, Yamlean, and Kojong 2013). Meanwhile, Sukrama et al. (2017) reported that concentrated Binahong leaf ethanolic extract effectively decreased the area of burn wounds in murine models (Sukrama, Wihandani, and Manuaba 2017). A study on excisional wounds in guinea pigs by Miladiyah and Prabowo (2012) showed that 40% Binahong leaf ethanolic extract in distilled water was efficacious in decreasing wound area (Miladiyah and Prabowo 2012). Histopathological studies on rats receiving 5% Binahong leaf extract showed a greater decrease in polymorphonuclear (PMN) infiltration and increase in collagen deposition, angiogenesis, and fibrosis in comparison to silver sulfadiazine (Yuniarti and Lukiswanto 2017).

Three main bioactive compound classes in Binahong leaves are known to play important roles in the wound healing process, namely saponin, tannin, and flavonoid (Leliqia, Sukandar, and Fidrianny 2017; Yuniarti and Lukiswanto 2017). Saponin enhances wound healing process through stimulation of procollagen synthesis. Saponin also enhances the proliferation of monocytes, which will differentiate into macrophages and secrete various growth factors. In the reepithelialization process, saponin stimulates fibroblast proliferation and keratinocyte migration (Yuniarti and Lukiswanto 2017). Fibroblasts will also secrete growth factors, such as VEGF, interleukins, and TGF-β (Sukrama, Wihandani, and Manuaba 2017; Sutrisno et al. 2018). Flavonoid and tannin are aromatic compound classes known for their astringent properties. The astringent properties of flavonoid and tannin compounds cause the skin pores to contract, hence stopping capillary bleeding and exudation and stimulates wound contraction (Ibrahim et al. 2018). In addition, Flavonoid enhances wound

healing through stimulation of collagen matrix rearrangement, while tannin stimulates wound contraction through its role in fibroblast migration and proliferation (Budovsky, Yarmolinsky, and Ben-shabat 2015; Ibrahim et al. 2018; Yuniarti and Lukiswanto 2017).

No significant increase in serum TGF-β1 was observed in all treatment groups. A previous study suggested that low concentration povidone iodine (0.5%) administered topically on clean wounds could stimulate TGF-β secretion (Wang et al. 2017). Therefore, higher concentration of povidone iodine administered and wound infection might have significantly impaired povidone iodine's effects on TGF-β. Saponin content in Binahong leaves has been thought to indirectly increase TGF-β secretion through stimulating fibroblast and monocyte proliferation (Sukrama, Wihandani, and Manuaba 2017; Sutrisno et al. 2018). Harvesting conditions might have influenced the saponin content of Binahong leaves prior to extraction. A previous study quantified more saponin content in older Binahong leaves than younger ones (2.36 μg/mg and 1.37 μg/mg, respectively) (Hasbullah 2016).

Most previous studies on the role of saponin compounds in increasing TGF-β release had not specified the exact TGF-β isoform studied. Among all TGF-β isoforms, TGF-β1 has so far been considered more important as it is also the most abundant. TGF-β1 has been known to induce integrin expression from keratinocytes in the skin epidermis, which facilitates the migratory components of reepithelialization (Ibrahim et al. 2018). Recently, TGF-β3, another isoform of TGF-β, has also been reported to play an important role in the later stages of wound healing. A study in murine models reported that TGF-β1 and TGF-β3 affected cell cycle progression and cell migration differently, which showed clinically in the formation of scar tissues. Higher TGF-β1 concentration tended to cause scar tissue formation, while higher TGF-β3 concentration

tended to promote scarless wound healing (Lichtman, Otero-vinas, and Falanga 2016).

Binahong leaf extract's effect on fibroblast and macrophage proliferation might have enhanced secretions of other growth factors and mediators as well. Fibroblasts and macrophages secrete a plethora of growth factors and cytokines, such as interleukins (IL-1, IL-6, IL-11, IL-17, IL-18), TNF- $\alpha$ , IFN- $\gamma$ , VEGF, PDGF, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which have known roles in the wound healing process (Gonzalez et al. 2016; Zeinali, Rezaee, and Hosseinzadeh 2017). Previously, a study on rats with *Pseudomonas aeruginosa*-infected burn wounds showed an increase of IL-6 and VEGF concentrations after administration of Binahong leaf concentrated extract (Sukrama, Wihandani, and Manuaba 2017).

Our findings have reinforced existing evidence on the efficacy of Binahong leaf ethanolic extract administration in enhancing wound healing, hence further suggesting its possible clinical application both as a single wound management product and in combination with other agents. However, our current study's findings were limited in that serum TGF- $\beta$ 1 was assayed only before treatment and after day 14 of treatment, which corresponded with later phases of wound healing. Considering the vast influence of TGF- $\beta$  in all phases of wound healing, possible increases in TGF- $\beta$ 1 during the earlier phases of wound healing still needs to be determined in order to better comprehend the effects of Binahong leaf ethanolic extract administration on TGF- $\beta$ 1 secretion. Another limitation was that this study focused on a single isoform of TGF- $\beta$ , and the possible effects of Binahong leaf ethanolic extract on other TGF- $\beta$  isoforms remains to be elucidated.

## 5 CONCLUSION

In rats with *Staphylococcus aureus*-infected wounds, topical administration of Binahong (*Anredera cordifolia* (Ten.) Steenis) leaf extract for 14 days was efficacious in decreasing wound area but did not increase serum TGF- $\beta$ 1 levels. Further studies need to assess the effects of Binahong leaf ethanolic extract administration on TGF- $\beta$ 1 levels in accordance with each phase of wound healing and investigate the effects of Binahong leaf ethanolic extract on other isoforms of TGF- $\beta$ .

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