

IN VIVO EFFICACY OF TOPICAL BINAHONG (*ANREDERA CORDIFOLIA* (TEN.) STEENIS) LEAF ETHANOLIC EXTRACT ON TRANSFORMING GROWTH FACTOR-BETA 1 IN INFECTED WOUNDSRIZKI ANDINI NAWAWI^{1*}, MUHAMMAD TOTONG KAMALUDDIN², THEODORUS²¹Postgraduate Student, Biomedical Sciences Graduate Program, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia.²Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia. Email: rizki.nawawi@mail.ru

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ABSTRACT

Objective: This study's aim was to assess the efficacy of topical Binahong (*Anredera cordifolia* (Ten.) Steenis) leaf ethanolic extract administration on serum transforming growth factor-beta 1 (TGF- β 1) in infected wounds.

Methods: 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. There were 30 male Wistar rats aged 10–12 weeks with excisional wounds 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 14 days of treatment. Wound area and bacterial count were also recorded and analyzed. Data analysis was performed using computer software.

Results: Wound size and bacterial count were significantly decreased in treatment groups receiving topical Binahong leaf ethanolic extract. No significant increase in serum TGF- β 1 was observed in all treatment groups.

Conclusion: Topical administration of Binahong leaf ethanolic extract on rats with infected wounds for 14 days did not significantly increase serum TGF- β 1.

Keywords: *Anredera cordifolia*, Wound infection, Transforming growth factor beta, *In vivo*.

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INTRODUCTION

Wounds are defined as a disruption in the integrity of skin, mucosa, or organ tissues [1]. Impairments in the wound healing process may cause various complications, especially when inadequately treated [2]. Physiological wound healing is generally divided into 4 phases: Hemostasis, inflammation, proliferation, and tissue remodeling. These processes involve a complex interaction between the skin's cellular components through secretion of various growth factors and mediators [1,3].

Transforming growth factor-beta (TGF- β) is a pleiotropic growth factor with a vast involvement in all phases of wound healing. TGF- β is secreted by platelets, fibroblasts, and pro-inflammatory cells, such as macrophages [4,5]. TGF- β 's role in the wound healing process includes stimulation of collagen synthesis, angiogenesis, and keratinocyte migration [5,6]. TGF- β also induces epithelial-mesenchymal transition, which becomes clinically important especially at the later phases of wound healing to the formation of scars [3,7]. TGF- β has 3 isoforms, namely, TGF- β 1 to TGF- β 3. TGF- β 1 is the most abundant isoform quantified in humans [8].

The presence of bacterial burden and infections may significantly impair wound healing [2,6]. One of the most predominant pathogenic bacteria causing wound site infections worldwide is *Staphylococcus aureus* [9,10]. A multicenter study in three Indonesian hospitals reported the presence of *S. aureus* in 45.3% patients with infected wounds and associated skin and soft tissue infections [9]. *S. aureus* releases various virulence factors, which cause disturbances in the wound healing process by prolonging inflammation and inhibiting angiogenesis [11,12].

Topical therapy remains an important modality often used in wound management. Yet, most available topical therapies do not offer additional benefits with regards to wound healing [13]. While antibiotic usage is not regarded as a treatment strategy in treating infected wounds, topical antibiotics such as mupirocin, clindamycin, polymyxin, neomycin, and fusidic acid are still often used in the clinical setting. This had contributed to the rise of antimicrobial resistance in organisms causing wound infections [14]. There had been a shift toward the use of topical antiseptics, such as iodine preparation and chlorhexidine, considering their similar effectivity against pathogens, but antiseptic tolerance and resistance have also emerged as a consequence [14,15].

Recently, incorporation of natural products and their active compounds in the formulation of new wound management products had gained attention [16]. *Anredera cordifolia* (Ten.) Steenis (Binahong) is a vine from the *Basellaceae* family, which has been extensively used in traditional medicine of various nations around the world [17]. In Indonesia, Binahong leaves had been used traditionally to treat wounds and bacterial infections [17,18].

Numerous studies had reported the efficacy of *A. cordifolia* extract in wound healing, yet to the authors' knowledge there had been no data yet on the *in vivo* efficacy of *A. cordifolia* extract on TGF- β 1 concentrations in infected wounds. Therefore, this study's aim was to determine the efficacy of *A. cordifolia* ethanolic extract on increasing serum TGF- β 1 in rats with *S. aureus*-infected wounds.

METHODS

An experimental study, *in vivo*, was conducted at the Biotechnology Laboratory and Animal House, Faculty of Medicine, Universitas Sriwijaya, Palembang, from July to September 2020. The study

population was male Wistar rats. The study subjects were male Wistar rats aged 10–12 weeks, weighing 150–200 g. Thirty rats showing clinical signs of infection (sloughing, redness surrounding the wound area, granulation tissue formation) within 24 h since inoculation with *S. aureus* were included in this study. Ethical clearance had been approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Sriwijaya before the commencement of this study (Certificate No. 024/kepkrsmhfkunsri/2020).

Binahong leaf ethanolic extract preparation

Four hundred grams of dried Binahong leaves were obtained from Karangpandan, Tawangmangu, Central Java (elevation: 800 m above sea level). Extraction process was performed by maceration with 96% ethanol for 72 h, as described in the Indonesian Herbal Pharmacopoeia [19]. The extract was then filtered and concentrated by rotary evaporation. The concentrated extract was 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.

In vivo efficacy study

The rats had undergone 1 week of acclimatization before the experiment. Before wounding, the rats were anesthetized using ketamine, and their dorsal skin was depilated with scissors and depilatory cream. A 2 cm² circular excision wound was then made using scalpels and surgical scissors. A suspension of *S. aureus* ATCC 25923 containing 2×10^7 cfu was inoculated into the wounds and incubated for 24 h. Afterward, the rats were grouped into 5 treatment groups, each receiving negative control, three concentrations of Binahong leaf ethanolic extract salves, and positive control.

All groups received treatments twice daily for 14 days. Wound area and bacterial count were measured as baseline data. Debridement with sterile saline solution was performed daily before administration of treatments. Wounds were photographed and swabbed with sterile cotton swabs on days 4, 7, 10, and 14 of treatment. Wound area was measured using image processing software. Swabs were plated onto mannitol salt agar (LabM, Lancashire, UK) for bacterial counting. Serum samples were obtained twice, before treatment started and after the 14th day of treatment. Serum TGF- β 1 level was assayed using ELISA (MyBioSource, San Diego, CA, USA), following protocols specified by the manufacturer.

Statistical analysis

Homogeneity and normality of the data were assessed before statistical analysis. Statistical analysis for efficacy was assessed using paired t test for normally distributed data and Wilcoxon test for non-normally distributed data. Efficacy comparison between treatment groups was done using unpaired t test for normally distributed data and Mann-Whitney test for non-normally distributed data. Statistical significance was assumed at $p < 0.05$.

RESULTS AND DISCUSSION

Wound area was significantly decreased in all treatment groups (Table 1). Changes in wound area during the course of the 14-day

treatment are presented in Fig. 1. The treatment group receiving 10% Binahong leaf extract showed the most decrease in wound area. There was a significant efficacy difference between the 10% Binahong leaf extract group and negative control group ($p = 0.022$). Yet, there was no significant efficacy difference between the 10% Binahong leaf extract group and povidone iodine 10% group (Table 2).

Topical administration of Binahong leaf ethanolic extract in all concentrations effectively decreased wound area, and treatment with 10% Binahong leaf ethanolic extract showed best decrease in wound area. Our finding was similar to a study by Paju *et al.* in rabbits with incisional infected wounds, which reported the efficacy of 10% Binahong leaf ethanolic extract in wound healing [20].

At present, studies conducted on the efficacy of Binahong leaf extract in wound healing have reported various findings. Sukrama *et al.* reported that concentrated Binahong leaf ethanolic extract administered without vehicle effectively decreased the area of burn wounds in murine models [21]. A study on guinea pig models with excisional wounds showed that 40% Binahong leaf ethanolic extract in distilled water was efficacious in decreasing wound area [22]. Histopathological studies on rats receiving 5% Binahong leaf extract showed a greater decrease in polymorphonuclear infiltration and increase in collagen deposition, angiogenesis, and fibrosis in comparison to silver sulfadiazine [23].

Binahong leaves contain three main classes of bioactive compounds which have been known to play important roles in the wound healing process, namely, saponin, tannin, and flavonoid. 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 [23]. Fibroblasts will also secrete growth factors such as vascular endothelial growth factor (VEGF), interleukins (ILs), and TGF- β [4,21]. Astringent properties of flavonoid and tannin compounds cause the contraction of skin pores, which helps stop capillary bleeding and exudation. The skin contraction, in turn, also stimulates wound contraction [24]. Flavonoid also enhances wound healing through stimulation of collagen matrix

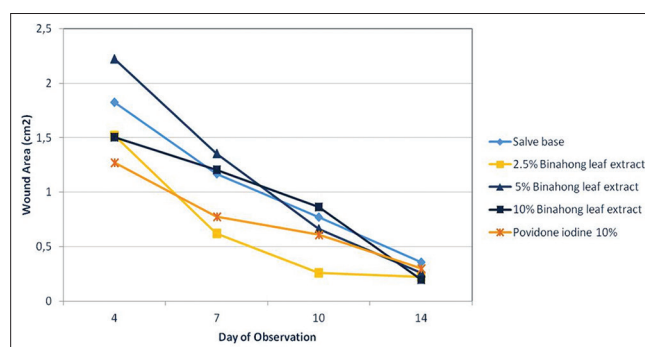


Fig. 1: Wound area contraction during the course of the experiment

Table 1: Efficacy of topical Binahong leaf ethanolic extract in decreasing wound area after 14 days of administration

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

Paired t test, $P = 0.05$

* $p < 0.05$, ** $p < 0.01$

rearrangement, while tannin further stimulates wound contraction through its role in fibroblast migration and proliferation [23-25].

Table 2: Efficacy comparison between different doses of topical Binahong leaf ethanolic extract and controls in 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 *p<0.05, **p<0.01

Table 3: Efficacy of topical Binahong leaf ethanolic extract in decreasing bacterial count after 14 days of administration

Treatment group	Bacterial count ($\times 10^8$ cfu)		p
	Pre-treatment Mean	Post-treatment Mean	
Salve base	51.571	19.286	0.018*
Binahong leaf extract 2.5%	42.200	22.000	0.225
Binahong leaf extract 5%	85.333	56.333	0.028*
Binahong leaf extract 10%	98.167	16.167	0.028*
Povidone iodine 10%	11.833	15.500	0.599

Wilcoxon test, P=0.05, *p<0.05, **p<0.01

Table 4: Efficacy comparison between different doses of topical Binahong leaf ethanolic extract and controls in decreasing bacterial count after 14 days of administration

Comparison group	Treatment group	p
Salve base (negative control)	2.5% Binahong leaf extract	0.807
	5% Binahong leaf extract	0.045*
	10% Binahong leaf extract	0.471
	Povidone iodine 10%	0.774
Povidone iodine 10% (positive control)	Salve base	0.774
	2.5% Binahong leaf extract	0.855
	5% Binahong leaf extract	0.030*
	10% Binahong leaf extract	0.748

Mann-Whitney test, P=0.05, *p<0.05, **p<0.01

Table 5: Efficacy of topical Binahong leaf ethanolic extract in increasing serum TGF- β 1 after 14 days of administration

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

Paired t test, P=0.05, *p<0.05, **p<0.01

Topical administration of Binahong leaf ethanolic extract decreased the bacterial count in the swounds (Table 3). The treatment group receiving 5% Binahong leaf ethanolic extract showed a significant efficacy in comparison to both negative and positive control (Table 4).

Flavonoid compounds contained in Binahong leaves had been known to form a complex with bacterial cell wall protein, damaging the cell wall and ultimately causing bacterial cell lysis [23]. Binahong leaf ethanolic extract also contains ursolic acid, which had been known to inhibit the growth of various pathogens, including *S. aureus* [26]. A previous study by Leliqia *et al.* determined the minimum inhibitory concentration of Binahong leaf ethanolic extract against various Gram-positive pathogens, including *S. aureus*. The authors reported that ethanolic extract of Binahong leaves obtained through maceration only has bacteriostatic activity against *S. aureus* [26].

After 14 days of topical Binahong leaf ethanolic extract administration, no significant increase in serum TGF- β 1 was observed in all treatment groups (Table 5).

Saponin content in Binahong leaves has been thought to indirectly increase TGF- β secretion through stimulating fibroblast and monocyte proliferation [4,21]. Harvesting conditions, especially the age of the leaves at harvest, might have influenced the saponin content of Binahong leaves before extraction. A previous study quantified more saponin content in older Binahong leaves (2.36 μ g/mg) than younger leaves (1.37 μ g/mg) [27].

Most previous studies on the role of saponin compounds in stimulating TGF- β release had not specified the exact TGF- β isoform studied. Among all TGF- β isoforms, TGF- β 1 has so far been considered more important as it is also the most abundant. TGF- β 1 has been known to induce integrin expression from keratinocytes in the skin epidermis, which facilitates the migratory components of reepithelialization [24]. Yet, recently, TGF- β 3, 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- β 1 and TGF- β 3 effects cell cycle progression and cell migration through initiation of different pathways, which showed clinical importance in the formation of scar tissues. Higher TGF- β 1 concentration tended to cause scar tissue formation, while higher TGF- β 3 concentration tended to promote scarless wound healing [5].

While we found no apparent increase in TGF- β 1 in this study, Binahong leaf extract's effect on fibroblast and macrophage proliferation might have enhanced wound healing by increasing the secretion of other growth factors and mediators. Fibroblasts and macrophages secrete a plethora of growth factors and cytokines such as ILs (IL-1, IL-6, IL-11, IL-17, IL-18), TNF- α , interferon gamma, VEGF, platelet-derived growth factor, and granulocyte-macrophage colony-stimulating factor, which have known roles in the wound healing process [28,29]. A study by Sukrama *et al.* on rats with *Pseudomonas aeruginosa*-infected burn wounds showed an increase of IL-6 and VEGF concentrations after administration of Binahong leaf concentrated extract [21].

Our study limitation was that serum TGF- β 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- β in all phases of wound healing, in this study, possible increases in TGF- β 1 during the earlier phases of wound healing might have not been observed and therefore missed for analysis.

CONCLUSION

Topical administration of Binahong (*A. cordifolia* (Ten.) Steenis) leaf ethanolic extract for 14 days did not increase serum TGF- β 1 levels in rats with infected wounds. However, topical Binahong leaf ethanolic extract administration did cause a significant decrease in wound area and bacterial count. Further studies need to assess the effects of Binahong leaf ethanolic extract administration on TGF- β 1 levels

in accordance with each phase of wound healing, and investigate the effects of Binahong leaf ethanolic extract on other isoforms of TGF- β .

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AUTHORS' CONTRIBUTIONS

R.A.N., M.T.K., and T. contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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