

Ethanollic extract of *Anredera cordifolia* (Ten.) Steenis leaves improved wound healing in guinea pigs

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ABSTRACT

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BACKGROUND

Wound healing is a normal biological process in response to skin injury. *Anredera cordifolia* (Ten.) Steenis is used traditionally to treat various diseases, including skin disease, hypertension, inflammation and gout. The aim of this study was to evaluate the wound healing activity of the leaves of binahong or *Anredera cordifolia* (Ten.) Steenis in guinea pigs.

METHODS

Thirty guinea pigs (1.5-2 kg, 3-4 months old) were randomly divided into 5 groups. Group I was given distilled water (negative control), group II was treated with povidone iodine 10% (positive control), while groups III-V were treated with ethanolic extract of binahong leaves at concentrations of 10%, 20%, and 40%, respectively. Before treatment, a 2 cm long excision wound was made on each animal. All interventions were given by the topical route, twice daily for 15 days. At the end of 15th day, the wound lengths in each group were measured and compared to baseline wound lengths. Data were analyzed with one-way Anova to compare wound healing activity between groups.

RESULTS

This study showed that groups treated with ethanolic extract of binahong leaves at concentrations of 20% and 40% experienced better wound healing activity than negative and positive controls. There were significant differences ($p=0.000$) between treatments and negative and positive controls.

CONCLUSIONS

This research has successfully show significance of the Binahong leaf extract has a potential for wound healing in guinea pig.

Keywords : *Anredera cordifolia* (Ten.) Steenis, wound healing, guinea pigs

Ekstrak etanol daun Anredera cordifolia (Ten.) Steenis (Basellaceae) memperbaiki penyembuhan luka pada marmut

ABSTRAK

LATAR BELAKANG

Penyembuhan luka merupakan suatu proses normal sebagai respon adanya cedera pada jaringan kulit. Secara tradisional *Anredera cordifolia* (Ten.) Steenis sering digunakan untuk mengobati berbagai jenis penyakit, termasuk penyakit kulit, hipertensi, peradangan dan gout. Penelitian ini bertujuan untuk menilai aktivitas penyembuhan luka daun binahong atau *Anredera cordifolia* (Ten.) Steenis terhadap luka eksisi buatan pada marmut.

METODE

Sebanyak 30 ekor marmut (umur 3-4 bulan, berat 1,5-2 kg dikelompokkan secara acak menjadi 5 kelompok perlakuan yaitu kelompok I diberi olesan akuades (kontrol negatif), kelompok II diberi povidone iodine 10% (kontrol positif), kelompok III-V diberi ekstrak etanol daun binahong dengan konsentrasi masing-masing 10%, 20%, dan 40%. Pada seluruh hewan uji dibuat luka eksisi sepanjang 2 cm, dan dioleskan perlakuan sesuai kelompoknya, dua kali sehari selama 15 hari. Pada akhir hari ke-15, diukur penyembuhan luka dari tiap kelompok (dalam persen, dibandingkan dengan luka awal). Data persen penyembuhan luka dianalisis dengan Anova satu jalan untuk melihat adanya perbedaan antar kelompok perlakuan.

HASIL

Studi menunjukkan bahwa ekstrak etanol daun binahong mampu menyembuhkan luka mulai konsentrasi 20%. Semakin tinggi konsentrasi ekstrak, efek penyembuhan luka semakin besar. Uji statistik menunjukkan bahwa pada kelompok ekstrak etanol konsentrasi 20% dan 40%, terdapat perbedaan signifikan dengan kontrol negatif (akuades) ($p=0,000$), maupun kontrol positif ($p=0,000$).

KESIMPULAN

Ekstrak etanol daun binahong mampu menyembuhkan luka lebih baik daripada povidone iodine. Daun binahong berpotensi pada penyembuhan luka.

Kata kunci : *Anredera cordifolia* (Ten.) Steenis., penyembuhan luka, marmut

INTRODUCTION

A wound comprises damage to anatomic structures and functions of the skin, thus resulting in loss of epithelial continuity with or without loss of surrounding connective tissue.⁽¹⁾ The effects of a wound may be in the form of partial or complete organ dysfunction, sympathetic activation, bleeding, bacterial contamination and cell death.⁽²⁾

The wound healing process is a normal biological response to injury, but the underlying biological factors are extremely complex. It is

a dynamic process, where the factors involved are regeneration and repair of tissue damage.⁽³⁾ Briefly, the wound healing process may be divided into 4 phases, i.e. (i) hemostasis, (ii) inflammation, (iii) proliferation, and (iv) remodelling.⁽⁴⁾ The remodelling phase is associated with the tensile or breaking strength of the skin, where 1 week after wounding the tensile strength is 3% of that of normal dermis, increasing within 3 weeks to 20%, and attaining after 3 months 80% of the breaking strength of normal dermis.⁽⁵⁾ The factors playing a role in wound healing include bacterial infection,

nutritional deficiencies, use of certain medications, obesity, movement of the wound site, and location of the wound.⁽⁶⁾ The surrounding tissues also affect the rate of healing, such as aseptic conditions, disposal of dead tissue, apposition of wound edges, and regular application of dressings.⁽⁷⁾ The utilization of traditional medicinal plants for wound healing is based on their antiseptic, adstringent, antiinflammatory, and antibacterial properties.⁽⁸⁾ The plants increase the rate of wound healing by supplying substances required in the tissue regeneration and proliferation phases,⁽⁷⁾ because they contain saponins, alkaloids, tannins, steroids, and glycosides.⁽⁶⁾ Among the plant parts used for wound healing, roots account for 27% and leaves 20%, while the remainder is made up of stems, seeds, whole plants, fruits, flowers, tubers, and other parts.⁽⁹⁾

One of the plants frequently used by local communities for wound healing is the *binahong* plant, its scientific name being *Anredera cordifolia* (Ten.) Steenis.^(10,11) The plant belongs to the Basellaceae family and is also known as *Boussingaultia gracilis* var. *pseudobaselloides* or *Boussingaultia baselloides*.⁽¹²⁾ It has been used by local communities for a number of healing applications, e.g. following surgery or delivery, and for diabetes and typhoid fever. The parts of the plant used for these purposes are the roots, stems, leaves, and flowers.⁽¹⁰⁾ Pharmacological tests conducted on *Anredera* spp. include the following: antibacterial,⁽¹³⁾ antiobesity and antihypoglycemic,⁽¹⁴⁾ cytotoxic and antimutagenic,⁽¹⁵⁾ antiviral,^(16,17) antidiabetic,⁽¹⁸⁾ antiulcer,⁽¹⁹⁾ and antiinflammatory.^(17,20) In vitro tests of wound healing activity is currently viewed to be inappropriate, since these tests are incapable of demonstrating the efficacy of a substance, such that animal experiments and human clinical trials are being increasingly used.⁽²¹⁾

Phytochemical analyses of *binahong* indicate that the leaves contain considerable amounts of saponins, alkaloids, and

flavonoids.⁽²²⁾ Thus far, there has been only one study conducted on the wound healing activity of *binahong*, involving hematomas in rats.⁽²³⁾ In the group receiving *binahong* there was macroscopic edema, but no infiltration of inflammatory cells on histopathological examination. The present study differs from the previous one in the plant parts used (leaves vs tubers), type of wound (excision wound vs hematoma), test animal (guinea pigs vs rats), and in the type of observation of the effects (macroscopic vs microscopic). Based on several preliminary studies, including studies on the phytochemical content of *binahong* leaves, the present study was performed to determine whether or not an ethanolic extract of the leaves of *Anredera cordifolia* (Ten.) Steenis showed wound healing activity on excision wounds in guinea pigs.

METHODS

Research design

This was a pure experimental controlled laboratory study to test the effect of *binahong* leaves on wound healing in guinea pigs.

Binahong leaves

Binahong leaves were obtained in April 2011 from the Kotagede region of Yogyakarta City, consisting of the fresh green leaves of plants that were at least 4 months old. Identification of the leaves was performed at the Plant Taxonomy Laboratory, Faculty of Biology, Gajah Mada University. Preparation of leaf extract was performed at the Pharmaceutical Biological Laboratory, Faculty of Mathematics and Natural Sciences, Indonesian Islamic University. The study was conducted at the Integrated Research and Testing Laboratory of Gajah Mada University.

Equipment and materials

Equipment and materials for the study were obtained from the last-mentioned laboratory, while povidone iodine for the

positive controls was from Kimia Farma. For anesthesia ketamine (Ketalar®) from Pfizer was used.

Preparation of *binahong* leaf ethanolic extract

Binahong leaf ethanolic extract was prepared by maceration as follows: *Binahong* leaves in the amount of approximately 1.5 kg was cleansed, cut up into small pieces and dried in a drying cabinet at 38°C for 5 days, then pounded and blenderized into a dry powder. Subsequently maceration was performed by putting the dry powder into an erlenmeyer flask containing 500 ml of 70% ethanol and leaving the mixture stand at room temperature for 24 hours. The maceration process was performed up to three times, after which the resulting solution (ethanolic phase) was filtered and concentrated in an evaporator at 50°C, yielding an ethanolic *binahong* leaf extract of 100%.

Experimental animals

The experimental animals used were 30 adult 3-4 months old guinea pigs weighing from 1.5 to 2 kg. The animals were obtained from the Integrated Research and Testing Laboratory of Gajah Mada University, with the provision of being in healthy condition (active and well-formed). Management and care of the animals before, during, and after the intervention was according to the standards of the Integrated Research and Testing Laboratory of Gadjah Mada University.

The numbers of test animals were based on previous studies, where with 5 types of intervention the minimum number of animals in each group was 5.⁽²⁴⁾ The use of guinea pigs in this study was based on the similarity of their characteristics to those of humans, with regard to general physiology of their cells, tissues and organs, thus being an appropriate animal model for several human diseases.⁽²⁵⁾

The test animals were assigned to intervention 5 groups, viz. negative controls (distilled water), positive controls (povidone

iodine 10%), and 3 groups treated with *binahong* leaf ethanolic extract at concentrations of 10%, 20%, and 40%, respectively (by dilution of the full extract with distilled water). The treatment consisted of topical application of the agent on the wounds, twice daily (morning and evening) for 15 days.

Determination of *binahong* leaf ethanolic extract concentrations

The determination of *binahong* leaf ethanolic extract concentrations was based on preliminary tests, where among the 5 concentrations used (5%, 10%, 20%, 40%, and 80%), all concentrations from 10% upwards had an effect.

Preparation of excision wounds

Before excision, the animals were anesthetized with Ketalar. The fur in the dorsal region was shaved, then a 2-cm long excision wound was made. During the study, the wounds were left open and exposed to the environment without topical or systemic antibiotic cover. Wound healing was recorded as the percentage of wound closure, calculated according to the formula: [(initial wound length – wound length on a given day)/initial wound length] x 100%.^(26,27)

Statistical analysis

The percentages of wound healing on day 15 were analyzed by one-way Anova (SPSS for Windows version 15), at significance level of $p < 0.05$.

RESULTS

Wound lengths were measured on days 6, 9, and 15, and converted to percentages of wound healing, as shown in Table 1. It is apparent that on the last day (day 15), wound healing in the group receiving *binahong* leaf extract 40% had attained 100%, which was higher than in the negative control group (59.17%), positive control group (68.33%),

Table 1. Mean percentages of wound healing on days 6, 9, and 15

Groups	Day 6	Day 9	Day 15	p
Distilled water	10.83 ± 5.85	25.00 ± 8.94	59.17 ± 18.28	0.000
Povidone Iodine	12.50 ± 2.74	24.17 ± 6.65	68.33 ± 13.66	
EEB 10%*	15.00 ± 4.47	33.33 ± 6.05	81.67 ± 4.08	
EEB 20%*	20.83 ± 5.84	45.30 ± 6.65	86.67 ± 16.63	
EEB 40%*	27.50 ± 5.24	50.00 ± 6.32	100.00 ± 0.00	

*EEB = Ethanolic Extract of *Binahong* Leaves

binahong leaf extract 10% group (81.67%) and *binahong* leaf extract 20% group (86.67%). Results of the Anova test indicated that these differences were significant ($p=0.000$), thus it may be stated that the ethanolic extract of *binahong* leaves was effective in wound healing. Post-hoc Anova showed that differences in wound healing expression were significantly differences ($p=0.000$) between *binahong* leaf extract 40% with Povidone iodine and *binahong* leaf 10% (Table 2).

DISCUSSION

Assessment of the effect of a drug on wound healing may be performed by means of various models. One of the models commonly used in experimental studies is the excision wound.^(6,26-31) This model is used to obtain information on duration of wound contraction and wound closure after administration of an extract in comparison to controls.⁽²⁶⁻²⁷⁾ The use of povidone iodine as positive control was based on previous studies,⁽³¹⁻³⁵⁾ from which it was assumed that the wound healing effect was due to the property of iodine as an effective broad-spectrum antimicrobial.⁽³⁶⁾

The *binahong* leaf ethanolic extract groups excellent results were found, where increasing the extract concentration led to better wound healing, as compared to the povidone iodine and the distilled water groups. Therefore it may be stated that *binahong* leaf ethanolic extract was effective in wound healing and better than povidone iodine 10%.

This study also shows that wound healing in the povidone iodine group gave no satisfactory results, being similar to the results in the negative control group. The use of povidone iodine as a topical drug on wounds is currently subject to debate among clinicians, because iodine may be systemically absorbed and thus cause damage and even toxicity in various tissues and cells,⁽³⁷⁾ including granulocytes, monocytes, and fibroblasts.⁽³⁶⁾ Povidone iodine is more effective as an antiseptic,⁽³⁷⁾ and thus more appropriately used as a wound dressing, particularly for infected wounds,⁽³⁸⁻⁴⁰⁾ and not for recent clean uninfected wounds. Therefore this study supports previous views that povidone iodine is not effective in wound healing.

This study demonstrates that administration of *binahong* leaf ethanolic

Table 2. Post-hoc Anova results on wound healing on day 15

	Akuades	Povidone iodine	EEB 10%	EEB 20%	EEB 40%
Distilled water	-	0.225 ^b	0.005 ^a	0.001 ^a	0.000 ^a
Povidone iodine	0.225 ^b	-	0.082 ^b	0.020 ^a	0.000 ^a
EEB 10%*	0.005 ^a	0.082 ^b	-	0.504 ^b	0.020 ^a
EEB 20%*	0.001 ^a	0.020 ^a	0.504 ^b	-	0.082 ^b
EEB 40%*	0.000 ^a	0.000 ^a	0.020 ^a	0.082	-

*EEB = Ethanolic Extract of *Binahong* Leaves; ^asignificant difference; ^bnon-significant difference

extract was capable of promoting wound healing to a high degree, which may be due to the anti-inflammatory, antioxidant, antibacterial, and analgesic effects of the extract.⁽⁸⁾ These effects may be caused by the saponin, alkaloid, and flavonoid content of *binahong* leaf ethanolic or aqueous extract.⁽²²⁾ A number of previous studies showed that the presence of saponins, alkaloids, polyphenols, flavonoids, glycosides, and triterpenes in the various parts of a plant may have a wound healing effect.⁽⁴¹⁻⁴⁷⁾

The mechanism of action of saponins in wound healing is to stimulate the production of type I collagen, which has an important role in wound closure and increases epithelialization of tissues.⁽⁴²⁾ Flavonoids act by inhibiting the lipid peroxidation process⁽⁴⁸⁾ and are responsible for free radical scavenging,⁽⁴⁵⁾ thus preventing and retarding cell necrosis, and increasing vascularization at the wound site. Inhibition of lipid peroxidation is believed to enhance the viability of collagen fibrils by increasing collagen fibers and vascularization, preventing cellular damage, and promoting DNA synthesis.⁽⁴⁸⁾ Flavonoids, glycosides, and tannins are known to act as adstringents and antibacterials.^(43,48) Polyphenols, being compounds with antioxidant properties, also play a role in wound healing, through inhibition of lipid peroxidation, as in the case of flavonoids.⁽⁴⁹⁾ The use of antioxidants in wound healing is due to the fact that cell proliferation, suppression of inflammation, and contraction of collagenous tissues are inhibited by the presence of free radicals.

It is the presence of saponins, alkaloids, and flavonoids in *binahong* leaves⁽²²⁾ that presumably plays a role in the wound healing process of the guinea pigs in the present study. However, which of these compounds is the most responsible for wound healing, has to be investigated in future studies, including testing of each of the isolated active compounds. A limitation of the present study is that it only involved macroscopic observation of the wound

healing effect, without in-depth investigations into the wound healing process and the cells involved.

CONCLUSIONS

Binahong leaf ethanolic extract is effective in the healing of excision wounds in guinea pigs. Povidone iodine is less effective in wound healing and should preferably not be used as a wound dressing, particularly in infected wounds.

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