

The effectivity of ethanolic extract of binahong leaves (*Anredera cordifolia* (tenore) steen) gel in the management of diabetic wound healing in aloxan-induced rat models

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ABSTRACT

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Background: Diabetes Mellitus (DM) is a disease that can be known by increasing a blood glucose level and caused many kinds of complications if it don't properly treatment, one of those complications is a diabetic ulcer. There are many types of treatments created to overcome the diabetic ulcer, but there are not effective yet. Therefore, ethanolic extract gel of binahong (*Anredera cordifolia* (Tenore) Steen) leaves is used to make a new innovation of diabetic ulcer treatment

Objective: The objective of this research was to know the concentration of antibacterial and anti-infection activity from ethanolic extract of binahong leaves as wound healing on diabetic ulcer and also to know the changeover of wound diameter.

Methods: Binahong leaves were extracted with 96% ethanol by maceration. Then the extract was formulated to be gel product with the concentration of 10% and 30%. The gel product was administered to diabetic rats which had been made ulcer wound by excision. The result of wound diameter and the percentage of wound healing were analyzed by One Way Anova and then continue analyzed by LSD (Least Significant Different) with significant level of 95%.

Results: The result showed that binahong gel with concentration variation of 10% and 30% only affected the organoleptic and doesn't affect the homogeneity, pH, irritation, spreadability and consistency. The result of the effectiveness test of binahong leaves gel is 10% more effective to changeover of wound diameter but there is not significantly different if compared with 30% gel of binahong leaves. Therefore, gel of binahong leaves of 10% is able to provide slightly effective than chloramphenicolTM.

Conclusion: The concentration of 10% and 30% of binahong gel were effectively usage for wound healing diabetic ulcer in rats

Latar belakang: Diabetes melitus merupakan suatu penyakit yang ditandai dengan meningkatnya kadar gula darah yang apabila tidak ditangani dengan baik akan menimbulkan berbagai komplikasi, salah satunya ulkus diabetik. Sudah banyak jenis pengobatan yang dilakukan untuk mengatasi ulkus diabetik, tetapi belum ada yang efektif. Oleh sebab itu dicari inovasi pengobatan baru dengan menggunakan gel ekstrak etanol daun binahong (*Anredera cordifolia* (Tenore) Steen).

Tujuan : Tujuan penelitian ini adalah mengetahui konsentrasi yang tepat terhadap aktivitas antibakteri

dan antiinfeksi ekstrak etanol daun binahong pada proses penyembuhan luka serta mengetahui perubahan diameter luka diabetes melitus.

Metode: Daun binahong diekstraksi menggunakan etanol 96% dengan metode maserasi. Kemudian ekstrak diformulasikan menjadi sediaan gel dengan konsentrasi 10% dan 30%. Gel tersebut diujikan pada tikus diabetes terinduksi aloksan yang dibuat luka eksisi infeksi. Hasil diameter luka dan persentase penyembuhan luka tersebut dianalisis dengan metode Anova satu arah (One Way Anova) dan dilanjutkan menggunakan LSD (Least Significant Different) dengan taraf signifikan 95%.

Hasil: Hasil penelitian menunjukkan gel daun binahong dengan variasi konsentrasi 10% dan 30% hanya berpengaruh terhadap organoleptis dan tidak berpengaruh terhadap homogenitas, pH, iritasi, daya sebar, dan konsistensi. Hasil uji efektivitas gel daun binahong 10% lebih efektif terhadap perubahan diameter luka tetapi tidak terdapat perbedaan yang signifikan apabila dibandingkan dengan gel daun binahong 30%. Selain itu, gel daun binahong 10% mampu memberikan efektivitas yang sedikit lebih baik dibandingkan kloramfenikolTM.

Kesimpulan: Dari data yang diperoleh, gel daun binahong 10% dan 30% efektif digunakan dalam menyembuhkan luka diabetes mellitus pada tikus.

INTRODUCTION

Indonesia is a country which has a lot of germplasm resources as raw materials for medicine. This may be one of the solutions to resolve the growing of various diseases that threaten a human life such as diabetes mellitus. Diabetes mellitus often cause a wound that are difficult to cure.

The skin disease which caused by diabetic wound is quite commonly found in Indonesia. Indonesia is one of 10 nations having the highest rate of diabetes mellitus, 8.5 million. This complication becomes a primary reason why the patients with diabetes mellitus have a prolonged of hospital stay. This is caused by the characteristic of the diabetic wound as a chronic healing. The prolonged wound healing is caused by late inflammation response. If the therapy uses a standard cure, the duration of wound healing will achieve 12-20 weeks. Now, there are

several studies using plant medicines in order to their benefit to healing a disease, especially to treat a wound. The height of interest from many researchers using the plants as medicine is caused by the assumption that the plant medicine is safer than synthetic drugs.¹ One of the plants which is known in Indonesia is Binahong (*Anredera cordifolia* (Tenore) Steen) family Basellaceae.

Binahong plant can accelerate the wound healing process by increasing the substances which is needed in tissue regeneration process and proliferation phase such as saponin, alkaloid, tannin, steroid, triterpenoid, flavonoid, ascorbic acid.² Based on those information, the idea to formulate of the ethanol extract of binahong leaves that can be used to treat skin infection has been raised. The formulation which will be made is in the form of a topical gel. Gel formulation known to be more efficiently used on wounds as it will not leak, give a cool sensation on the wound, do not leave marks on the wound after topical and easily absorbed.³

A research, was conducted by Yuliani (2012)⁴, concerning wound healing hydrogel dosage formulations of binahong leaves ethanol extract explained that the ethanol extract of binahong leaves with levels of 5% has the biggest wound healing activity of white blood cells with a value of 63.30%. Therefore, this study uses a variation of concentration of 10% and 30% in order to determine the effectiveness of the gel formulation of binahong leaves ethanol extract. The aim of this research was to evaluate the exact concentration of binahong ethanol extract on diabetic wound healing through determination of wound diameter which would affect on wound healing.

METHODS

This study was a true experimental method to evaluate the effectiveness of binahong leaves ethanol extract at concentration 10% and 30% on the diabetic wound. Wound healing was determined in male Wistar rats (160-250 g, 3-4 months). For this purpose, 25 rats were randomly divided into 5 groups. All experiments

were conducted in Biology Laboratory, Ahmad Dahlan University, Yogyakarta, Indonesia for two months, January to February 2015. This study was approved by Ethics Committee of Ahmad Dahlan University (KEP UAD), Yogyakarta.

Preparation of binahong leaves ethanol extract gel

Materials which were used in this study were dry simplisia. 400 g of Binahong simplisia were blended, extracted with 1500 mL of 96% ethanol, stirred for one hour using shaker water bath at 120 rpm, macerated as long as 24 hours at room temperature, and filtrated using Buchner funnel after 24 hours. Residue filtration was then remacerated for 24 hours. This experiment was repeated up to 3 times. The result of residue filtration of 1 and 3 was mixed and concentrated with rotary vacuum evaporator at 50°C.⁵ In this research, gel preparation made in various concentration: 10% and 30% as much as 25 g to 28 times usage for 14 days observations.

In this research, gel preparation made in various concentration which is 10% and 30% as much as 25g to 28 times usage for 14 days observations. The Formulation of ethanolic extract of binahong leaves gel 10% were Binahong leaves ethanol extract 2,5g, Na-CMC 1,25g, Glycerin 2,5g, Propylene glycol 1,25g, Aquadest ad 25g. The Formulation of ethanolic extract of binahong leaves gel 30% were Binahong leaves ethanol extract 7,5g, Na-CMC 1,25g, Glycerine 2,5g, Propylene glycol 1,25g, Aquadest add 25 g.

The extract was dissolved in water and boiled at 50°C. Then., Na-CMC, glycerine, propylene glycol and water were added and stirred until homogenized gel formulation was obtained. Finally, the gel was stored in the dark and cold room at 10°C -15°C for one night.⁶

Evaluation of gel preparation

Organoleptic test :The physical appearance, odor, and color of the gel formulations were studied by visual observations.⁷ Homogeneity test: Gel homogeneity test was conducted on a glass plate, touched and visualized. Observations

were made on day 2, day 4, and day-to-6. PH test: Gel was measured by using a universal pH stick dipped in gel sample that has been diluted, the result is adjusted to pH universal standard. The requirement of skin preparations pH is in the interval 4.5-6.5.⁸ Skin irritation test: Skin irritation was conducted on rats 0.5 grams of gel was applied to the back of three rats that had been shaved (backs of mice skin must not hurt). Observations of irritation test performed at 24 hours and 72 hours after the gel had applied to the backs of mice.⁸ Dispersive power test: Gel as much as 0.5 grams was placed in the middle of a round glass scale. Another round glass transparent and 150 grams weight were placed on that of glass scale, allowed to stand for 1 minute, and the diameter of dispersion. A good dispersive power gel is between 5 to 7 cm².⁹ Consistency test: Consistency test was conducted by centrifuge of gel at 3800 rpm for 5 hours. The separation between the excipients and gelling agent.¹⁰

Giving a Dose of Alloxan: a dose of 150 mg/kg alloxan monohydrate was induced to the rats intraperitoneally. On the third days, the blood glucose levels were measured.¹¹ The rats which were used for this test were rats with elevated blood sugar levels over 300 mg / dL.¹²

Creation of wound

Wounds creation was conducted in the control group, chloramphenicol group, binahong Gel 10% and 30% three days after the induction of alloxan. The normal group was not induced by alloxan but the rats still wounded on the third day. Before wound creation, the glucose levels was measured, ketamine HCl at a dose of 0.5 ml/kg was given intravenously, and a pattern with a circle diameter of 1.5 cm was created. The epidermal skin was cut on the pattern that had made. The wound creation must not injury the part of the muscle. The wound was left until that wound caused an infection with the characteristics as follows redness, yellowish pus, smelled and looked wet.¹³

Animal Experiments

This study was used 25 rats which were divided into 5 groups. The experiment that would be done as follows: Treatment A: rats were not induced alloxan, only cleaned the wound with 0.9% NaCl [normal group]. Treatment B: alloxan-induced mice intraperitoneally with a dose of 150 mg/kgbw. Treatment C: alloxan-induced mice intraperitoneally with a dose of 150 mg / kg b.w, the wound was cleaned with 0.9% NaCl and smeared with chloramphenicol cream [™], 2 times a day [Chloramphenicol group]. Treatment D: alloxan-induced mice intraperitoneally with a dose of 150 mg/kg BW, the wound was smeared with binahong leaves extract 10% and cleaned with 0.9% NaCl, 2 times a day [Binahong Gel 10% group]. Treatment E: alloxan-induced mice intraperitoneally with a dose of 150 mg/kg b.w, the wound was smeared with gel binahong 30% extract and cleaned with 0.9% NaCl, 2 times a day [Binahong Gel 30% group].

Diameter Measurement Process

The diameter of the wound was done by measuring of wound diameter vertically, horizontally, and two diagonally.¹³ Measurements were done one daily a day which was calculated by $d \times (1,2,3)$: the average of wound diameter/ each re-intervention, d: amount of intervention. Calculated by using the formula:

$$P\% = \frac{(d_0 - dx)}{d_0} \times 100\%$$

The average of diabetic wounds of each animals: In addition, to determine the extent of the wound healing process, measurement was done by calculating the percentage of wound healing. Calculation formula: $P\% = (d_0 - dx) / d_0 \times 100\%$. (P%: the percentage of wound healing; d_0 : the diameter of initial wound; dx: the diameter of the wound on the days of observation).

Analysis Statistic

The data that obtained was analyzed statistically using SPSS 16.0. One Way ANOVA analysis was used to know the difference between the interventions. If the data has not normal distribution or the variance of data is not the same, Kruskal-Wallis test will be used. One-way ANOVA, to analyze whether the gel that

has been made has a healing effect on diabetic wound, was based on calculated F value, F table and p value. If calculated F was less than F table, the gel of binahong leaves ethanol extract in each intervention would not have effect on diabetic wound healing. If calculated F was more than F table, the gel of binahong leaves ethanol extract in each treatment would have a healing effect on diabetic wound. If the Anova test showed a significant result, p value <0.05, there were the differences in diabetic wound healing effect in each treatment. Finally, the analysis would be proceed using of post-hoc LSD (Least Significant Different) to determine differences in significant treatment groups.¹⁴

RESULTS

The results of gel evaluation

The results of organoleptic test showed that Binahong gel was dark green colored, had Binahong leaves unique scent, had bitter taste, and had soft consistency. The results of homogeneity test of all samples showed good homogeneity. The results of pH measurement was 6,5 which means that all gels being produced met the criteria for skin usage. The results of irritation test for both gel samples showed no rash or erythem, itchiness, and swelling of the skin. The results of dispersivity test showed that the dispersivity of the gels with Na-CMC base had fulfilled all the parameters for adequate dispersivity.

Blood Glucose Measurement

The results of blood glucose concentration measurement in day-0, day-3 and day-23 of each group showed significant difference ($p < 0,05$) among intervention groups except for normal group (Table 1). It was then continued with LSD test to determine the difference significancy among groups.

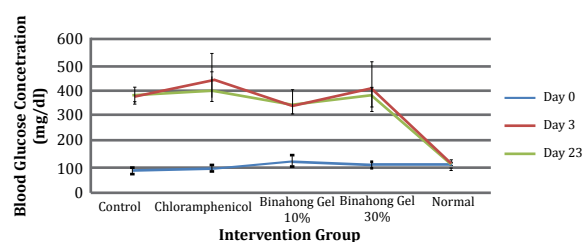


Figure 1 Complete Blood Glucose concentration (day-0, day-3 and day-23)

Table 1. Mean of Complete Blood Glucose Measurement

Group	Mean ± SD (mg/dl)		
	Day -0	Day -3	Day-23
Control	85,2 ± 6,06 ^a	381,2 ± 41,76 ^c	375,6 ± 48,22 ^b
Chloramphenicol	93,4 ± 12,07 ^a	437,2 ± 122,07 ^c	396,4 ± 81,79 ^b
Binahong Gel 10%	128 ± 32,60 ^a	348 ± 44,54 ^c	345 ± 44,55 ^b
Binahong Gel 30%	111,2 ± 20,17 ^a	415 ± 105,33 ^c	375,4 ± 51,70 ^b
Normal	108,6 ± 5,55	104 ± 8,49	104,4 ± 19,77

Notes : Different notation showed significant difference of each intervention groups every day.
Confidence interval 95%, p = 0,011

Table 1 of the intervention group showed significant increased of blood glucose from day -0 to day-3 and day-0 to day-23. This is coherent with the data of blood glucose concentration in Table I of the intervention group where the mean of animal models blood glucose concentration before being induced with aloxan in day-0 was approximately 82,75 ± 2.99 mg/dl to 115,0 ± 1,73 mg/dl. The in day -3 post aloxan induction, the mean of blood glucose concentration became 331,0 ± 15,56 mg/dl to 486,0 ± 8,49 mg/dl. And in day-23 the mean of blood glucose still became 312 ± 8,49 mg/dl to 396,5 ± 2,12 mg/dl.

DM Parameters

The result of symptoms accompanying DM include: wate intake, body weight, food intake and Feed Efficiency Ratio (FER). The measurement of water or drink intake was done before intervention, which was on day-0, and until intervention was done, which was on day-1 until day -23. Data showed an increase of water intake in control, chloramphenicol, binahong gel 10%, and binahong gel 30% group. While normal group only had minimal water intake increased (Table 2 and Figure 2).

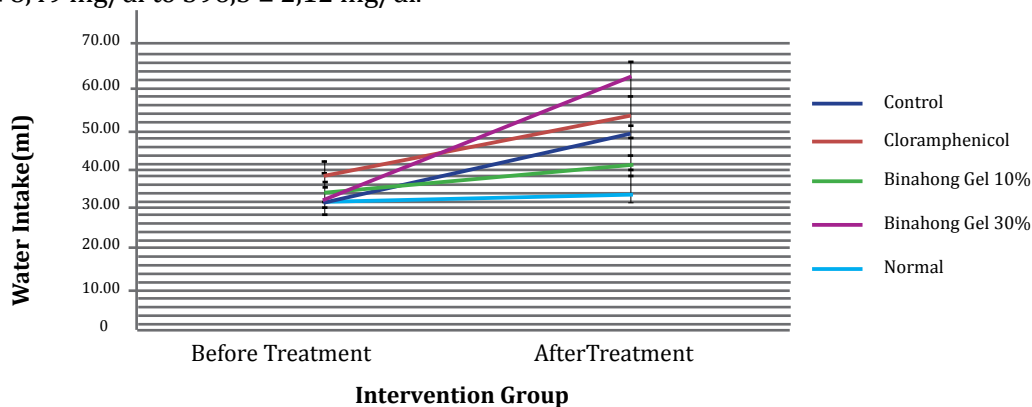


Figure 2. Mean of water intake before and during intervention

Table 2. Data of water intake measurement

Measurement	Mean ± SD (ml)				
	Control	Chloramphenicol	Binahong gel 10%	Binahong gel 30%	Normal
Before intervention	31,00 ± 1,00	38,67 ± 3,21	34,6 ± 4,13	32,2 ± 4,51	32,37 ± 2,9
During intervention	50,13 ± 4,49	53,75 ± 4,55	41,03 ± 4,88	61,46 ± 3,68	33,89 ± 3,6

The body weight showed decrease body weight in control, chloramphenicol, binahong gel 10%, and binahong gel 30% group. While the normal group showed increase in body weight (Figure 3). Food Intake group ($p < 0,05$) with the measurement of pre-excision wound compare to measurement on intervention day -19. Figure 4

showed statistically significant increase of food intake in every intervention.

Feed efficiency ratio is the relationship between rat models body weight and food intake. The result of feed efficiency ratio measurement could be seen in Figure 5.

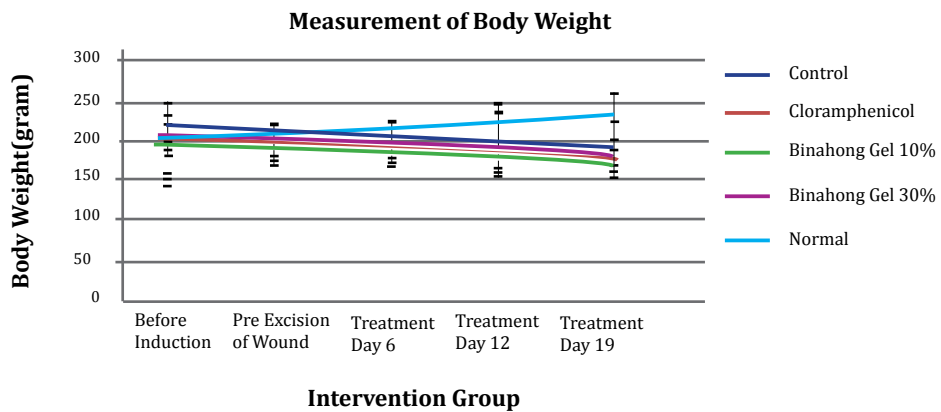


Figure 3 Body weight changes of rat models during research period

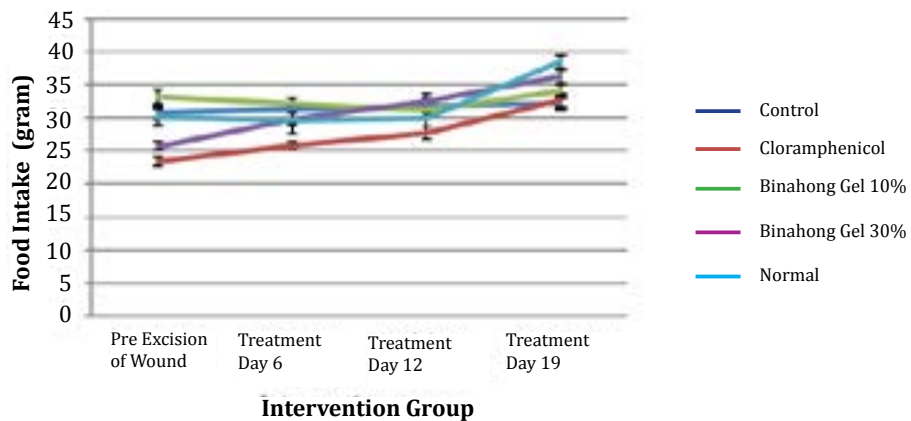


Figure 4 The mean of food intake during research period

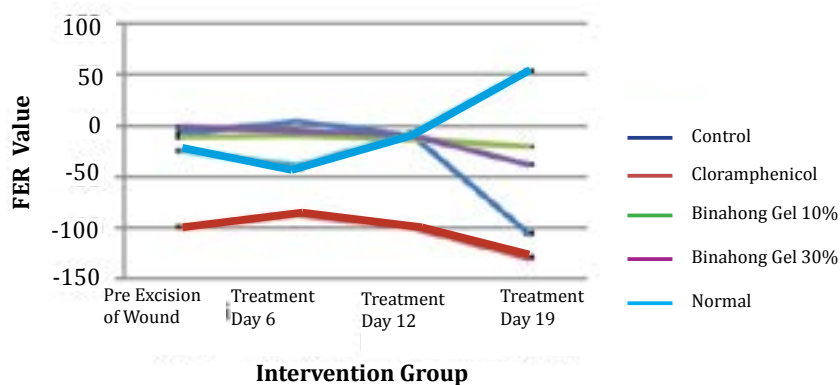


Figure 5 Feed Efficiency Ratio (FER)

Wound Diameter Measurement

The results of mean wound diameter measurements of every intervention group were statistically analyzed with One Way ANOVA and continued with LSD test. Statistical analysis

showed significant measurement results of each group among every measurement periods ($p < 0,05$) (Table 3 and Figure 7). The illustration of wound diameter measurement

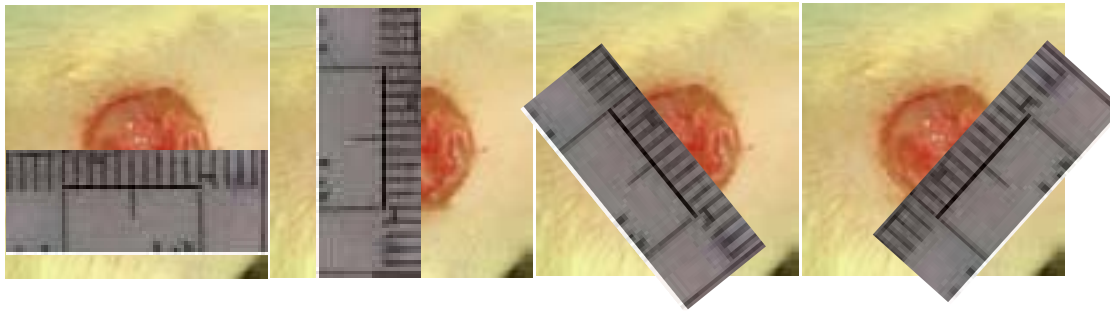


Figure 6. The illustration of wound diameter measurement

Table 3. The results of Wound Diameter Measurement in Day -1 to Day-19

Day-	Mean of wound diameter \pm SD (cm)				
	Control	Chloramphenicol	Binahong gel 10%	Binahong gel 30%	Normal
1	1,5 \pm 0,04	1,5 \pm 0	1,5 \pm 0,05	1,6 \pm 0,09	1,6 \pm 0
6	1,6 \pm 0,06	1,6 \pm 0,08	1,6 \pm 0,18	1,7 \pm 0,20	1,7 \pm 0,12
12	1,0 \pm 0,17	0,9 \pm 0,19	0,6 \pm 0,08	0,7 \pm 0,14	0,9 \pm 0,21
19	0,7 \pm 0,13 ^e	0,4 \pm 0,08 ^d	0,3 \pm 0,08 ^a	0,2 \pm 0,03 ^b	0,5 \pm 0,04 ^c

Notes : Different notation showed significant difference

Confidence interval 95%, $p = 0,000$

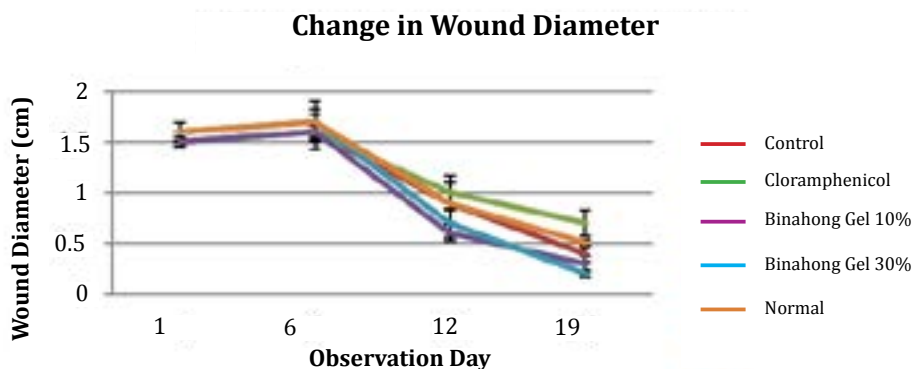


Figure 7 Wound diameter measurements graphic

The percentage of wound healing in every group was also statistically analyzed with One Way ANOVA and then continued with LSD test.

The results showed statistically significant difference of every groups between measurement periods ($p < 0,05$) (Table 4).

Table 4. The percentage of wound healing in each group

Day-	Percentage of wound healing \pm SD (%)				
	Control	Chloramphenicol	Binahong gel 10%	Binahong gel 30%	Normal
1	0,00 \pm 0	0,00 \pm 0,04	0,00 \pm 0,05	0,00 \pm 0,09	0,00 \pm 0
6	-6,67 \pm 0,08	-6,67 \pm 0,06	-6,67 \pm 0,18	-6,25 \pm 0,20	-6,25 \pm 0,12
12	33,33 \pm 0,19	40,00 \pm 0,17	60,00 \pm 0,08	56,25 \pm 0,14	43,75 \pm 0,21
19	53,33 \pm 0,08	73,33 \pm 0,13 ^d	80,00 \pm 0,08 ^b	87,50 \pm 0,03 ^a	68,75 \pm 0,04 ^c

Notes : Different notation showed different significancy between control group with intervention group (a= control and binahong gel 10%, b= control and binahong gel 30%, c= control and normal, d = control and chloramphenicol) with Confidence Interval 95%, p = 0,0001

Table 5. The result of LSD test Wound Healing Percentage

Comparison of Intervention Group		Sig.	Std. Error
Chloramphenicol : Control	Control : Chloramphenicol	0,810	
Chloramphenicol : Binahong Gel 10%	Binahong Gel 10% : Chloramphenicol		
Chloramphenicol : Binahong Gel 30%	Binahong Gel 30% : Chloramphenicol	0,781	
Chloramphenicol : Normal	Normal : Chloramphenicol	0,997	
Control : Binahong Gel 10%	Binahong Gel 10% : Control	0,631	27,18336
Control : Binahong Gel 30%	Binahong Gel 30% : Control	0,605	
Control : Normal	Normal : Control	0,812	
Binahong Gel 10% : Binahong Gek 30%	Binahong Gel30% : Binahong Gel 10%	0,970	
Binahong Gel 10% : Normal	Normal : Binahong Gel 10%	0,807	
Binahong Gel 30% : Normal	Normal : Binahong Gel 30%	0,778	

Statistical analysis showed significant difference of the percentage of wound healing ($p < 0,05$) between binahong gel 10% and 30% group with control group. This means that both gel was effective to be used in diabetes mellitus wound management. However, there was no statistical significant difference between binahong leaves gel in the concentration of 10% and 30% ($p > 0,05$). The same result was also seen in binahong gel 10% and 30% with chloramphenicolTM. The result of wound healing percentage analysis using LSD test showed no statistically significant difference ($p > 0,05$) between each intervention group.

DISCUSSION

The higher the extract concentration, the stronger the unique scent produced. The darker the green color, the bitter the taste produced.

The dark green color produced by the gel of binahong leaves extract in the concentration of 10% and 30% might be caused by a high chlorophyll content, while the bitter taste might be caused by the alkaloid content of binahong leaves.¹⁵ Hence, the difference in concentration of the ethanolic extract of binahong leaves in the concentration of 10% and 30% would affect the organoleptic aspect of the gels.

According to Garg, et al⁹, the adequate dispersivity of a gel should be between approximately 5-7 cm. The gel samples already met this criteria, in which the dispersivity of the gel was found to be ± 5 cm. However, this finding was only within the minimally adequate dispersivity capability. This was probably caused by the viscosity of Na-CMC which was too thick. When Na-CMC was inserted into the water, Na⁺ detached and was substituted by H⁺ ion and

formed HCMC that would increase viscosity.¹⁶ The result of consistency test of all gel samples showed that all gels could maintain stability and was not affected by gravitation for 1 year storage, because no separation was found.¹⁰

Results of water intake showed that control, chloramphenicol, binahong gel 10%, and binahong gel 30% group had diabetes even until the end of intervention period, coherent with general DM symptoms which would easily feel thirsty or also called polydipsia.¹⁷ The result of water intake measurement of each group showed statistically significant difference with ($p < 0,05$).

Body weight of control, chloramphenicol, binahong gel 10%, and binahong gel 30% group was in diabetic condition even until the end of intervention, which is coherent with diabetic theory that would show decrease in body weight.¹⁷ The result of body weight measurement before intervention until intervention day-19 showed no statistically significant difference of mean body weight ($p > 0,05$).

The significant increase of food intake occurred in all intervention group. This is due to variations of each rat models condition. Generally, DM patients would easily feel hungry or have increase appetite, also called polyphagy.¹⁷ Feed efficiency ratio is the comparison between the number of efficient feed intake between normal and abnormal condition. In normal condition, FER would be high, in which the increase of body weight would be in line with food intake. This is different in diabetic condition, in which FER would be low. Contrary with normal condition, body weight would decrease even with high food intake.¹⁸ Control, chloramphenicol, binahong gel 10%, and binahong gel 30% group showed low FER value. Hence, this data could be used as a supporting parameter to make sure that rat models was in diabetic condition even until the end of intervention period.

Statistical analysis showed significant difference of wound diameter between binahong gel 10% group with control group. This result indicated that gel in the concentration of 10% was effective enough to be used in diabetes mellitus wound management. However. It could

be seen in the data above that the group who had the most decrease of wound diameter on day -19 was the binahong gel 30% group, in which the mean of wound diameter was 0,2 cm. However, when compared with binahong gel 10% group, it was not statistically significant ($p < 0,05$) because the difference was only 0,1 cm. The lowest diabetic wound healing capability was seen in control and normal group. This was because no medical intervention or active ingredients administration was given to these two group, thus no diabetic wound healing assistance was provided. In these condition, wound was only cleaned with normale saline or NaCl 0,9% solution that has no bactericidal or bacteriostatic properties, and could only decrease the number of microorganism.¹⁹ In addition to that, control and normal group also showed decrease wound diameter in rat models. This finding means that human body has natural capability to protect and heal itself.²⁰ However, the healing process in control group was found more difficult than in normal group, probably because the wound in control group was more prone to infection which was caused by high blood glucose concentration that would inhibit wound healing.

CONCLUSION

Ethanollic extract gels of binahong leaves in the concentration of 10% and 30% could provide best capability in order to heal diabetic wound. The variation of ethanollic extract of binahong leaves 10% and 30% only affect organoleptic, but did not affect homogeneity, pH, irritation, dispersivity, and consistency. On day-19, wound that was intervened with binahong gel 10% showed decrease in diameter into 0,3 cm. While wound that was intervened with binahong gel 30% showed decrease in diameter into 0,2 cm. Statistical analysis showed that these difference were not significant, thus binahong gel 10% was found more effective in healing diabetic wound economically and more efficient to produce. In addition to that, binahong gel 10% showed better effect compare to chloramphenicol™.

SUGGESTION

Further research is needed in order to determine the best formulation and decolorophyllization to reduce the dark green color from the gel.

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