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Antioxidant Gel from Brown Algae (*Ascophyllum nodosum)* and Binahong Leaves for Diabetic Wound Healing

Salma Rahmadianti,[1] Irine Sofianty,[1] Lisa Aslamiah,[2] Febrian Khoirul Insan,[1] Maria Engzelita Sihombing,[2] Vidia Afina Nuraini,[1] Hernawati Hernawati[2]

[1]Study Program of Chemistry, Department of Chemistry Education, Universitas Pendidikan Indonesia, Indonesia, [2]Study Program of Biology, Department of Biology Education, Universitas Pendidikan Indonesia, Indonesia

Abstract

Background: Diabetic wounds are difficult to treat, causing persistent infections and often leading to limb amputation. One convenient way to manage diabetic wounds is by applying wound dressings. This study aimed to develop a wound dressing in form of a gel containing brown algae and binahong leaf extract for diabetic wound dressings.

Methods: This study was an experimental study conducted in the Organic Chemistry Laboratory of Universitas Pendidikan Indonesia from June to September 2022. As many as 33 mice induced by alloxan were divided into 9 groups consisting of application of brown algae extract gel, binahong leaf extract gel and a mixture of brown algae, and binahong leaf extract gel with the concentration of 2.5%, 5%, and 10% respectively. All treatments were given within 7 days. Laboratory data collected included phytochemical screening, total phenolic compound, antioxidant activity and organoleptic test.

Results: Brown algae and binahong leaf extracts had a high total phenolic content of 331.25 mg GAE/g and 207.01 mg GAE/g, respectively with antioxidant activity of IC_{50}= 327.33 µg/ml and 209.30 µg/ml, respectively. It was found that brown algae and binahong leaf extracts could accelerate wound closure in a diabetic mouse model. Gel formulation with 10% w/w brown algae extracts presented 91.66% of wound closure, two times greater than treatment with commercial drugs.

Conclusion: The developed gel containing brown algae and binahong leaf extracts can be a promising wound dressing for healing diabetic wounds.

Keywords: Antioxidants, binahong leaves, brown algae, diabetic wounds, gel

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Correspondence:
Vidia Afina Nuraini, S.Si., M.Sc, Study Program of Chemistry, Department of Chemistry Education, Universitas Pendidikan Indonesia, Indonesia
E-mail:
v.nuraini@upi.edu

Introduction

The International Diabetes Federation (IDF) in 2021 stated that as many as 537 million people have diabetes worldwide. This number is predicted to increase to 643 million in 2030 and 783 million in 2045. In 2021, Indonesia is ranked fifth with the most diabetes cases in the world, namely 19.5 million people and is expected to increase to 28.6 million people in 2045. Diabetes mellitus is a disease characterized by high blood sugar levels[1] and is highly associated with various comorbidities such as heart damage, blood vessel diseases, kidney failure, and neuropathy in the wounds or ulcers.[2]

Diabetic foot ulcer is one of the most common complications of diabetes, leading to amputation in 15–25% of patients and suffering from chronic disability. Cotton gauze has been used in wound dressings to protect diabetic wounds. However, these dressings are not suitable for diabetic wounds because of painful secondary damage.[3] Besides, many studies have developed gels with antioxidant properties that can make more favorable conditions for wound healing. This is because antioxidant gel can reduce excess reactive

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oxygen species (ROS) in chronic wounds so that it can accelerate wound healing.[4] Low levels of ROS can stimulate cell migration and good angiogenesis for wound healing, but excessive amounts of ROS can inhibit chronic wound healing.[4] These characteristics indicate that the gel can be used as a wound dressing.

One natural source of antioxidants is algae. Algae is one of the commodities that is easily cultivated in Indonesian waters but its existence has not been optimized. Algae has been widely studied because apart from containing bioactive compounds, it also produces free radicals and other antioxidant compounds when in conditions with high oxygen concentrations so that the algae does not experience structural damage because it is able to produce antioxidant compounds that can protect itself from oxidation.[5]

All studies on algae suggest that brown algae has a higher phenolic content and has more potential as an antioxidants when compared to green and red algae.[6] Binahong leaves also contain antioxidants. Binahong leaves contain saponins, tannins, terpenoids, alkaloids, and flavonoids. Saponin has a role as an antiseptic which can prevent bacterial growth in wounds.[7] Tannins and alkaloids have antioxidant and antimicrobial properties which can inhibit the growth of pathogenic bacteria in wounds.[8] Flavonoids act as anti-inflammatory, antioxidant and anti-aging properties. and are able to inhibit the activity of enzymes involved in bacterial metabolism.[8] This study aimed to obtain the best formula for brown algae and binahong leaf extract as an antioxidant gel, determine the characteristics of the resulting gel, and determine the effectiveness of the antioxidant gel in the diabetic wound healing process.

Methods

This study was an experimental study and was carried out at the Organic Chemistry Laboratory of the Universitas Pendidikan Indonesia, in June–September 2022. This study consisted of 3 steps, the first was extraction of brown algae (*Ascophyllum nodosum*) and binahong leaves (*Anredera cordifolia*), the second step was making a formula antioxidant gel and did several tests to analyze the extract and gel content; the third was pre-clinical testing in diabetic mice.

Brown algae (*Ascophyllum nodosum*) was extracted as much as 150 g using a mixture of water/ethanol (50:50, v/v) (1,500 ml) in a glass bottle. Extraction was carried out in an ultrasonic bath at room temperature for 30 minutes, then centrifuged at 3000× g for 10 minutes at 4 °C. The filter was filtered using vacuum filtration. The extract was dried and stored at –20°C.[9]

Binahong leaves were macerated as much as 320 g using 95% ethanol for 24 hours at room temperature. After 24 hours, the liquid was removed and filtered. The residue was soaked again in 95% ethanol and macerated again, then the final filtering process was carried out. A rotary evaporator was used to evaporate the solvent until the extract became dry.[10]

Determination of total phenolic content (TPC) was carried out using visible light spectrophotometry with Folin Ciocalteu reagent. The basis of this method was the formation of a blue complex detectable at a wavelength of 760 nm. The TPC values of the three extracts were expressed in Gallic acid equivalent (GAE) mg/g extract. A total of 75 mg of each type of extract was dissolved little by little in 50 ml of distilled water. For each type of extract solution, 0.5 ml was added to 2.5 ml of Folin-Ciocalteu 10% reagent. After that, 2 ml of 20% Na2CO3 was added to the solution. Gallic acid standards were prepared for comparison with concentration of 3.125, 6.25, 12.5, 50, and 100 ppm.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent solution was prepared by dissolving 4 mg of DPPH in 25 ml methanol, keeping it under light, and storing it in a refrigerator. Samples of brown algae extract, binahong leaves, and a mixture of brown algae and binahong leaves were diluted to concentrations of 1,000, 100, 50, 12.5, 6.25, and 3.125 ppm respectively, 4 ml of which was dissolved in 1 ml of DPPH. Solutions of each extract and DPPH were incubated in the dark and at room temperature. The UV-Vis spectrophotometer was used for measurements at a wavelength of 517 nm.[10,11]

Phytochemical tests were carried out on samples of binahong leaves, brown algae, and a mixture of binahong leaves and brown algae. In the flavonoid test, 1 ml of the extract sample was put into a test tube, then 2 drops of 10% sodium hydroxide (NaOH) were added and shaken vigorously (positive for flavonoids if they produces a yellow, red, or brown color).[12] In the alkaloid test, 2 ml of the extract sample was put into a test tube plus 2 ml of concentrated hydrochloric acid (HCl) and 5 drops of Mayer's reagent (positive for alkaloids if it produces a white precipitate).[13] In the coumarin test, 1 ml of the extract sample was put into a test tube

Figure 1 (A) Binahong leaves (*Anredera cordifolia*), (B) Brown Algae (*Ascophyllum nodosum*)

plus 1 ml of 10% NaOH (positive for coumarin if it produces a yellow color). In the phenol test, 2 ml of the extract sample was put into a test tube plus 5 ml of ethanol, and two drops of 2% FeCl3 were added (positive for containing phenol if it produced a dark blue color).[14]

The gel base used was expanded in 20 ml of distilled water for 24 hours. Carbopol, gelatin, and carboxy methyl cellulose (CMC) were put into the beaker slowly while stirring. Glycerol and propylene glycol were added while stirring until homogeneous then water was added until the weight of the mixture was close to 95 g. After that, the mixture was sterilized using a beaker covered with aluminum foil and stored in an autoclave at 121°C for 15 minutes.[15] Sterile binahong leaf extract and brown algae were added according to formulation of 2.5, 5.0, and 10.0%. The antioxidant gel formulation was shown in Table 1.

Organoleptic tests included observing the color, odor, and texture of the gel. In order to test the dispersion, 0.5 g of gel was placed on a slide and then covered with another slide, then the dispersion was measured using a ruler from the center point of the slide. After the first measurement, the slide was given a load of 50 g for 1 minute and measured. The pH test on the gel was measured using a pH meter by dipping it into the sample.

This study used male mice (*Mus musculus*) as test animals because mice were easy to handle and male mice did not have the hormone estrogen or the amount of estrogen hormone was very small.[16] The number of samples was calculated based on the number

Table 1 Formulation of Binahong Leaf Extract Gel, Brown Algae, the Mixture of Binahong Leaf with Brown Algae, and Base

Gel Type	Sample (%)								
	A1	A2	A3	B1	B2	B3	C1	C2	C3
Brown algae extract	2.5	5.0	10.0	-	-	-	-	-	-
Binahong leaf extract	-	-	-	2.5	5.0	10.0	-	-	-
Brown algae + binahong leaf	-	-	-	-	-	-	2.5	5.0	10.0
Carbopol	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Gelatin	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
CMC	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Propylene glycol	2	2	2	2	2	2	2	2	2
Glycerol	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
TEA	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Note: CMC= Carboxymethyl cellulose, TEA= Triethanolamine

Table 2 Phytochemical Test Results

No	Test parameters	Extract sample		
		Binahong Leaf	Brown Algae	Mixture Binahong Leaf+Brown Algae
1	Flavonoid	+	+	+
2	Alkaloid	–	–	–
3	Coumarin	+	+	+
4	Phenol	–	–	–

Table 3 Results of Organoleptic Tests, Dispersion Tests, and pH Tests

Variable		Organoleptic Test			Dispersion Test	pH
Extract	Concentration	Color	Odor	Texture		
Binahong leaf	2.5%	Thin green	Slight scent	Liquid	6.7	9.32
	5%	Slightly dark green	Scent	Slightly liquid	6.8	9.25
	10%	Dark green	Strong scent	Slight viscous	7.0	8.06
Brown algae	2.5%	Thin brown	Scent	Slight viscous	6.1	9.37
	5%	Slightly dark brown	Strong scent	Viscous	6.0	9.08
	10%	Dark brown	Very strong scent	Very viscous	5.3	9.83
Mixture of binahong leaf and brown algae	2.5%	Thin greenish brown	Scent	Liquid	5.7	9.34
	5%	Slightly greenish brown	Scent	Slightly liquid	5.5	9.11
	10%	Dark greenish brown	Strong scent	Viscous	5.0	8.98

of groups in the study using the Federer formula. Based on these results, this study was conducted with 3 repetitions for each treatment. The characteristics of the mice used were 5–6 months old with a body weight of 25–30 g and the total number of mice used was 33. After adaptation, mice were fasted for 16–18 hours. Then, the blood sugar level was measured using a glucometer content. Each mouse was induced with 150 mg/kg alloxan intramuscularly. The test animals were then fed and given water again. After three days of administering alloxan, blood sugar levels (hyperglycemia) were measured.[17] The data obtained was analyzed statistically using the One Way ANOVA method to see whether the gel made had a healing effect on diabetic wounds based on the calculated F values and F tables. This study has received approval from the Research Ethics Committee Universitas Padjajaran with number 897/UN6.KEP/EC/2022.

Results

The results of the TPC, DPPH, and phytochemical tests were carried out on extracts of brown algae and binahong leaves. The results of TPC determination showed that binahong leaf extract had a higher TPC value than brown algae. Standard gallic acid was made for the TPC test which aimed to determine the actual value of gallic acid at a certain concentration and determine the equation of the curve line. The equation of the curve line was y=0.0096x + 0.0032 with R2= 0.9992. The phenol content was determined per ml with the y-axis being the absorbance value and the x-axis being the average phenol content. The TPC of brown algae (Ascophyllum nodosum) and binahong leaves were 331.25 mg gallic acid equivalent (GAE)/100 g and 207.01 mg GAE/g, respectively.

DPPH radical was an organic compound containing unstable nitrogen with strong absorbance at a wavelength of 517 nm. The antioxidant activity of the sample resulted in a color change from purple to yellow. This change occured when DPPH free radicals were captured by antioxidants and release hydrogen atoms to capture stable DPPH-H. Each extract sample showed an increase in % inhibition per concentration. The percentage of inhibition was obtained from the difference between the absorbance of the control and the sample. Inhibitory Concentration 50% (IC_{50}) value of brown algae extracts was 327 µg/ml and binahong leaf extract was 209.30 µg/ml.

The phytochemical test showed the extract of binahong leaves and brown algae contained

Table 4 Glucose Level Test Results

Sample	Sugar Level (mg/dl)		Sample	Sugar Level (mg/dl)		Sample	Sugar Level (mg/dl)	
	Initial	End		Initial	End		Initial	End
A1a	34	117	B1a	73	161	C1a	73	165
A1b	86	139	B1b	79	107	C1b	114	132
A1c	64	121	B1c	76	117	C1c	44	158
A2a	66	102	B2a	59	174	C2a	95	159
A2b	86	120	B2b	76	120	C2b	86	166
A2c	77	136	B2c	82	158	C2c	58	130
A3a	100	154	B3a	76	189	C3a	45	154
A3b	98	147	B3b	84	135	C3b	131	163
A3c	107	123	B3c	75	145	C3c	82	121
K(+)a	73	134	K(-)a	65	130			
K(+)b	62	125	K(-)b	88	150			
K(+)c	57	130	K(-)c	79	142			

Note: A1= Brown algae with a concentration of 2.5%, A2= Brown algae with a concentration of 5.0%, A3= Brown algae with a concentration of 10.0%, B1= Binahong leaf with a concentration of 2.5%, B2= Binahong leaf with a concentration of 5.0%, B3= Binahong leaf with a concentration of 10.0%, C1= Binahong leaf + brown algae with a concentration of 2.5%, C2= Binahong leaf + brown algae with a concentration of 5.0%, C3= Binahong leaf + brown algae with a concentration of 10.0%, K(-)= Mice without treatment, K(+)= Mice treated with commercial drugs, a-c= Repetition

flavonoid and coumarin compounds. Negative results for alkaloids were shown because due to the absence of white precipitates using Mayer's reagent (HgCl2+KI). The phenol test yielded a negative result (Table 2).

In the organoleptic test, there were differences in color and texture in binahong leaf, brown algae, and mixture of binahong leaf and brown algae extract with different concentrations. Meanwhile, the dispersion test and pH test showed numbers that were slightly different for each extract (Table 3).

The results of observations of mice blood glucose levels after acclimatization for 7 days showed an increase in sugar levels after alloxan induction (Table 4). Testing the effectiveness of diabetic wound healing showed that the wound closure process took 6 days and the progress of wound healing in each mouse was observed for 6 days by using the gel once a day (Table 5).

Discussion

This study describes the potential of brown algae and binahong leaves extract as an effective gel in healing diabetic wounds. In the total phenol test, phenol compounds are chemical compounds that may act as antioxidants. The total phenol yield, the total phenol contained in brown algae was very large, namely 331.25 mg GAE/100 g and higher than binahong leaves. Previous study has evaluated the type of brown algae *Ascophyllum nodosum* which has a higher total phenol than other types of brown algae, namely 4.66 g phloroglucinol equivalents (PGE)/100 g dry weight (DW).[9] Meanwhile, another study found that dried binahong leaves (*Anredera cordifolia*) yielded 54.4 mg GAE per 100 g of dry matter (DM) (mg GAE /100 g DM).[18] The total phenolic in binahong leaves on plant-environment interactions play an important role. The amount of polyphenols can change according to the phenological stage, plant development, and environmental conditions. Seasonal effects on *A. cordifolia* leaves cause variations in phenolic content and antioxidant activity.[19] As the leaves age, the phenolic content decreases. Young leaves of *A. cordifolia* have a faster metabolic response to thermal stress, resulting in greater accumulation of phenolics. Younger tissues have been shown to have higher rates of secondary compound biosynthesis.[20]

In the antioxidant test, antioxidants are defined as substances that can prevent free radical autoxidation reactions in lipid oxidation.[21] Based on the antioxidant activity results, the antioxidant activity of binahong leaves is greater with a lower IC_{50} value than brown algae. The low IC50 value indicates a strong ability of the extract to act as a hydrogen atom donor.[22] Previous study stated that if a material contains phenolic compounds, the antioxidant activity of that material is also high.[10] In this study, the high antioxidant activity value in brown algae extracts was

Table 5 Clinical Trials of Mice

Sample	Wound Length (cm)					Mean ±SD	Average Percentage
	Day 2	Day 3	Day 4	Day 5	Day 6		
A1a	0.6	0.5	0.4	0.3	0.2	0.4 ± 0.1	
A1b	1.0	1.0	0.9	0.3	0.3	0.7 ± 0.3	78.88
A1c	0.5	0.7	0.4	0.0	0.0	0.3 ± 0.3	
A2a	1.0	1.0	0.9	0.2	0.3	0.7 ± 0.3	
A2b	0.7	0.7	0.5	0.3	0.2	0.5 ± 0.2	74.92
A2c	0.6	0.6	0.5	0.1	0.1	0.4 ± 0.2	
A3a	0.8	0.5	0.5	0.2	0.2	0.4 ± 0.2	
A3b	0.7	0.6	0.3	0.2	0.3	0.4 ± 0.2	91.66
A3c	0.6	0.6	0.4	0.0	0.0	0.3 ± 0.3	
B1a	0.6	0.6	0.3	0.3	0.0	0.4 ± 0.2	
B1b	0.7	0.8	0.8	0.4	0.3	0.6 ± 0.2	72.38
B1c	0.5	0.5	0.2	0.0	0.2	0.3 ± 0.2	
B2a	0.7	0.6	0.4	0.2	0.1	0.4 ± 0.2	
B2b	0.6	0.5	0.4	0.4	0.3	0.4 ± 0.1	61.90
B2c	0.8	0.6	0.5	0.5	0.4	0.6 ± 0.1	
B3a	0.6	0.8	0.5	0.6	0.6	0.6 ± 0.1	
B3b	0.6	0.5	0.2	0.0	0.0	0.3 ± 0.2	54.16
B3c	0.8	0.8	0.4	0.4	0.3	0.5 ± 0.2	
C1a	0.7	0.5	0.2	0.0	0.0	0.3 ± 0.3	
C1b	0.5	0.5	0.4	0.2	0.2	0.4 ± 0.1	77.14
C1c	0.7	0.7	0.4	0.0	0.2	0.4 ± 0.3	
C2a	0.8	0.8	0.5	0.5	0.4	0.6 ± 0.2	
C2b	1.0	0.6	0.5	0.1	0.0	0.4 ± 0.4	79.63
C2c	0.9	0.6	0.3	0.2	0.1	0.4 ± 0.3	
C3a	0.7	0.6	0.3	0.1	0.0	0.3 ± 0.3	
C3b	0.5	0.5	0.3	0.1	0.1	0.3 ± 0.2	86.66
C3c	1.0	1.0	0.7	0.3	0.2	0.6 ± 0.3	
K(+)a	0.9	0.7	0.6	0.4	0.4	0.6 ± 0.2	
K(+)b	0.7	0.6	0.5	0.5	0.3	0.5 ± 0.1	45.99
K(+)c	0.8	0.7	0.7	0.6	0.6	0.7 ± 0.1	
K(-)a	0.8	0.8	0.6	0.3	0.3	0.6 ± 0.2	
K(-)b	1.0	0.9	0.8	0.6	0.6	0.8 ± 0.2	43.69
K(-)c	0.7	0.8	0.5	0.5	0.5	0.6 ± 0.1	

Note: A1= Brown algae with a concentration of 2.5%, A2= Brown algae with a concentration of 5.0%, A3= Brown algae with a concentration of 10.0%, B1= Binahong leaf with a concentration of 2.5%, B2= Binahong leaf with a concentration of 5.0%, B3= Binahong leaf with a concentration of 10.0%, C1= Binahong leaf + brown algae with a concentration of 2.5%, C2= Binahong leaf + brown algae with a concentration of 5.0%, C3= Binahong leaf + brown algae with a concentration of 10.0%, K(-)= Mice without treatment, K(+)= Mice treated with commercial drugs, a-c= Repetition

negatively correlated with the high total phenol content in the algae. Antioxidant activity is not only caused by phenolic compounds. Other compounds that can act as antioxidantsare pentacyclic triterpenes, vitamin C, and dyes such as chlorophyll, sulfur compounds, and nitrogen.[23] Previous studies have evaluated the brown algae *Ascophyllum nodosum*, which has a DPPH value of 50 mol TE/g DW, and the leaves of binahong (*Anredera cordifolia*) which yields an IC50 value of 87.42 g/ml.[9, 24]

The wavelength determination was carried out using a methanol blank. The maximum methanol absorbance obtained was 0.0315 in the brown algae extract test and 0.036 in the binahong leaf extract test at a wavelength range of 517 nm. Furthermore, the maximum wavelength of DPPH was used to determine the antioxidant activity of each extract. The maximum wavelength of DPPH used in

binahong leaf extract is 0.8864. Meanwhile, the maximum wavelength of DPPH in brown algae extract is 1.2699. Before determining the DPPH free radical scavenging activity of each extract, a calibration curve was made to test the linearity of the samples. A quantitative test was carried out to determine the free radical reducing activity expressed by the IC50 value. The IC_{50} value is defined as the concentration of the test compound that can reduce free radicals up to 50%.

In photochemical tests, the extract of binahong leaves and brown algae contained flavonoid compounds. These results are in accordance with previous studies which stated that binahong leaf extract contains flavonoids compounds.[25,26] Binahong leaves contain coumarin which has a therapeutic effect on diabetes and its complications through repairing pancreatic beta cells, increasing insulin signaling, as well as anti-inflammatory and antioxidant.[27] For alkaloids, the extract solution does not change and there is no white precipitate because no potassium-alkaloid complex is formed. Phenol tests with negative results can occur due to the temperature and heating time during extract preparation so it is not identified qualitatively.

The gel formulated in this study is made with a cooling sensation and moisturizes the wound area to reduce swelling, thus speeding up the healing process by reducing pain in the wound. The gel was made using carbopol, gelatin, CMC, propylene glycol, glycerol, and triethanolamine (TEA) bases. Carbopol, gelatin, and CMC were used as gelling agents which were proven to be effective in producing good preparations. Propylene glycol and glycerol function as surfactants and co-surfactants agents respectively which can reduce surface tension, increase gel flexibility, do not cause irritation, and have low toxicity compared to alcohol. The TEA base functions as a pH buffer.[15]

Based on the organoleptic tests, the greenish color is the result of the binahong leaf extract content and the brown color is the result of the brown algae extract content. There was a color change from the colorless gel base to greenish in the binahong leaf extract and brown in the brown algae extract. The concentration level consists of 2.5, 5.0, and 10.0%. The higher the concentration level, the more distinctive the aroma of binahong leaves and brown algae. In the texture test, the higher the concentration, the thicker the gel.

The dispersion test was carried out to determine the ability of the gel to spread. The spreadability or dispersion of each extract formulation mets the requirements, namely between 5–7 cm.[28] Based on the results, the greater the concentration of binahong leaf extract, the higher the spreadability. Likewise with the mixture of binahong leaf extract with brown algae, the greater the concentration of the mixture of binahong leaf extract with brown algae, the higher the spreadability. Meanwhile, for brown alga extract, the greater the concentration of brown algae extract, the lower the spreadability. The gel with binahong leaf extract is more dilute than the brown algae extract and affects the dispersion obtained.

Gel of binahong leaves extract, brown algae, and a mixture of binahong leaves with brown algae do not meet the pH requirements of the skin, which are in the range of 4.5–6.5. If the pH is not within this range it can cause skin irritation.[29] This can be caused by the addition of TEA which is an alkalizing agent. The addition of a slight excess of TEA is thought to cause the pH of the preparation to be more than 6.5. Dosage usage should be investigated further to prevent irritation.

Clinicals trial have been used to evaluate the effectiveness of diabetic wound healing. The average value of negative controls or wounds without gel preparation treatment was 43.69%. Meanwhile, the average percentage of healing in the positive control using commercial gel preparations was 45.99%. Based on the data from this study, all gel formulas of binahong leaf extract, brown algae, and a mixture of binahong leaves with brown algaehave an effect on healing diabetic wounds. The formulation with the best healing percentage was sample A3 or gel with brown algae extract with a concentration of 10.0%. In binahong leaf extract, the higher the concentration, the smaller the healing percentage. When compared to the healing percentage values with positive controls or commercial drug treatments, the healing effects of brown algae extract and binahong leaf formula are more effective and have high value.

The data obtained was analyzed statistically and the results showed that F count >F table with the an F count value of 20.08 and an F table value of 1.55, so the binahong leaf extract gel, brown algae, and a mixture of binahong leaves with brown algae for each treatment had a healing effect on diabetic wounds.

Although this study has shown significant results from the TEA addition. However, there are several other inevitable drawbacks. The main problem was that the pH of each formula was higher than expected which was

caused by the addition of TEA as an alkalizing agent. Another thing is that this study only analyzed wound healing in mice based on the length of the wound. Some methods such as dermatological tests and histological analysis have not been carried out.

In conclusion, this study shows that the developed gel containing brown algae and binahong leaf extracts can be a promising wound dressing for healing diabetic wounds. Future study regarding gel formulations need further exploration to obtain the best formula for healing diabetic ulcer.

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