

ORIGINAL ARTICLE

The Effect of Brown Algae (*Sargassum Sp*) Extract on Burns Wound Healing

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ABSTRACT

Background: The wound care method that is currently developing uses the principle of moisture balance which is also known as the modern dressing method. Seaweed is a potential commodity in Indonesia, which has been widely used as a medicinal ingredient. However, the use of seaweed in the form of hydrogel ointment in the treatment of burns has not been widely studied. Therefore, this study was conducted to determine the effect of giving seaweed extract (*Sargassum sp.*) in the form of hydrogel ointment to burns.

Methods: The design in this study was an experimental study with a randomized post-test only control group design, using *musmusculus* mice. This study was divided into 4 groups, group 1 was a negative control without treatment, group 2 was a group with burns and was given bioplacementon, group 3 was a burn group treated with 5% *Sargassum sp* extract hydrogel ointment and group 4 was a burn group given 5% ointment. *Sargassum sp* extract hydrogel ointment 10%. The treatment was carried out for 14 days and evaluated the diameter of the burn and the percentage of burn healing.

Results: The results of this study showed that the difference in burn diameter at 0 days and after 14 days in the negative control group experienced a difference of 1.2 cm with a healing percentage of 44.85%. In treatment group 1, there was a difference in diameter of 1.2 cm, with a healing percentage of 49.41%, the 2-day treatment group obtained a difference in diameter of 1.2 cm, with a healing percentage of 50.31%, and in the positive control group a wound diameter of 1.0 cm, while the percentage of cure is 55.75%. The results of statistical tests on the percentage of wound healing between groups were not significantly different.

Conclusion: In this study the seaweed extract (*Sargassum sp.*) formed in the hydrogel ointment did not affect the healing of burns, this may be due to the long duration of administration.

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Introduction

Wounds are a serious problem in world health, especially in developing countries. The World Health Organization (WHO) reported that in 2004, the incidence of injuries worldwide averaged 110/100,000 people each year and an estimated 310,000 people died from injuries such as burns, infected wounds, etc. In 2015, approximately 486,000 injuries occurred in the United States, 40,000 of which required hospitalization and 300,000 requiring treatment in wound care centers.¹

In Indonesia, there are no exact figures regarding injuries, but with the increase in population and industry, the number of injuries is increasing. Hospital Dr. Soetomo has a wound care center, the number of burns that are treated at RSUD Dr. Soetomo Surabaya since 2007-2011 as many as 665 occurred.²

Burns are skin damage due to excessive heat or chemicals.³ There are three types of burns, namely first degree, second degree and third-degree burns. The three types of burns, third degree burns are burns that can destroy all layers of the skin.⁴

The wound healing process requires time and proper care so that the wound can heal quickly. Wound care methods that are currently developing are using the principle of moisture balance which is also known as the modern method of dressing. Modern wound care must still pay attention to three stages, namely washing the wound, removing dead tissue and choosing a dressing. Conventional wound care must often replace gauze with wound dressings, while modern wound care has the principle of

maintaining wound moisture, one of which is hydrogel material.⁵

Hydrogel functions to keep the wound moist, soften and destroy necrotic tissue without damaging healthy tissue, which is then absorbed into the gel structure and wasted with the dressing.⁴ The basic ingredients of hydrogels are glycerin or water so that they can provide moisture.

Seaweed is a potential commodity in Indonesia, seaweed production in Indonesia has increased significantly with an increase of 76.4 percent from 5.2 million wet tons of seaweed in 2011 to 9.2 million tons in 2013. Seaweed is one of the ingredients in wound dressing. Seaweed contains bioactive compounds such as flavonoids, saponins, tannins, albumin, which are useful as antibacterial, anti-obesity, cholesterol-lowering, anti-inflammatory, immunostimulant, antioxidant.⁶

Several studies on the use of seaweed as wound dressings for wounds have also been carried out, such as the research of Annisa et al, 2018 on the effect of brown algae extract *Sargassum sp* on healing of traumatic ulcers.⁷ And by Aprinaldi's research, 2020 regarding the effectiveness test of red seaweed on wound healing.² As well as previous study from Mutia in 2011 regarding the use of alginate membranes from *Sargassum sp* seaweed as wound dressings and delivery of topical drugs.

From several studies on the use of seaweed as a topical wound medicine, there has never been any research on the use of seaweed formed in hydrogel ointment and its effectiveness for burns. So as to add to the scientific study on the use of seaweed, this research was conducted to determine the effect of

seaweed extract (*Sargassum sp.*) which is formed in the hydrogel ointment on the main wound, which is burns.

Methods

Animal Model

This research was conducted in the research laboratory and experimental animal laboratory, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya. Ethical approval was granted by the animal ethics committee of Universitas Nahdlatul Ulama Surabaya with the number 182/EC/KEPK/UNUSA/2021.

The design in this study is an experimental study with a randomized post-test *only control group design*. The population of the research sample is adult white male mice (*Mus musculus*), the age is between 2-3 months and the weight of the mice is 20-30 grams. During the study mice were given standard feed. Mice in healthy condition were characterized by active and not isolated movements in the corner of the cage, clean and smooth fur, clear eyes, no abnormal discharge from the eyes, ears, anus and no defects and no weight loss of up to 10% during acclimatization.

Extract of *Sargassum sp*

Sargassum sp obtained from Lampung, Sumatra. *Sargassum sp* was weighed as much as 250 grams and put into an erlenmeyer, then added solvent until the final volume reached 1000 ml with a ratio of 1: 4 (w/v). The extraction procedure was carried out by the maceration method by immersing the sample with 90% ethanol. The maceration results were then filtered with *Whatman 42* filter paper to produce

filtrate and residue. The filtrate obtained was then concentrated with a *vacuum rotary evaporator* at a temperature of 40 °C to obtain a crude extract in the form of a paste.⁸

Sargassum sp extract paste that has been finished is ready to be made in the preparation of hydrogel ointment. The preparation of *Sargassum sp* ointment hydrogel preparations is processed by mixing the basic gel ingredients, namely carbopol 940, methyl paraben, propyl paraben, TEA, glycerin, propylene glycol and aquadest and added *Sargassum sp* extract. The composition of the hydrogel preparation ingredients is in the following Table 1.

Experimental Design

This study was divided into 4 groups, namely a negative control group, a positive control group, and 2 treatment groups. The number of samples was calculated based on the Federer formula: $(n-1) (t-1)$ 15. Each group had 6 mice, so the total number of samples was 24 mice. The negative control group was the group that was treated with wounds and plain ointment hydrogel without *Sargassum sp* extract. The positive control group was the group that was treated with wounds and given *Bioplacenton*. Treatment group 1 was the group that was treated with wounds and given a 5% hydrogel ointment with *Sargassum sp* extract. Treatment group 2 was treated with wounds and was given 10% ointment hydrogel *Sargassum sp* extract.

Statistical analysis

All results were expressed as mean \pm SEM. The unpaired Student's ttest was performed to compare of

parameters between two groups . Comparisons of dose-response curves were made by two-factor repeated measures ANOVA, followed by Tukey's post hoc test for comparison between groups. A value of $P < 0.05$ was considered significant.

Table 1. Formulation of Hydrogel Ointment

Ingredients	Formulation (%)			
	Plain	<i>Sargassum sp</i> 5%	<i>Sargassum sp</i> 10%	Bioplacenton
<i>Sargassum sp</i> extract	0%	5%	10%	0%
Carbopol 940	4 %	4 %	4 %	0 %
Triethanolamine	4 %	4 %	4 %	0 %
Glycerin	20 %	20 %	20 %	0 %
Propylene glycol	10 %	10 %	10 %	0 %
Methyl paraben	0,4 %	0,4 %	0,4 %	0 %
Propyl paraben	0,4 %	0,4 %	0,4 %	0 %
Aquadest Ad.	Ad. 100	Ad. 100	Ad. 100	0 %

The process of making burns in mice is carried out on the back area by shaving and cleaning the skin first. Then disinfection with alcohol. Mice were previously anesthetized using ketamine + acepromazine 100/5 mg/kg bw intramuscularly. Burns are made with an iron plate. the diameter of the iron plate is 2 cm. an iron plate that has been heated on a blue fire for 3 minutes then affixed to the back skin of mice for 5 second.⁹

After 14 days, the mice were euthanized using ether. Burn healing was evaluated for 14 days by measuring the diameter of the burn. The data from the diameter measurement of burns on the first day and the last day were analyzed using the formula (9):

$$D\% = \frac{d_0 - dx}{d_0} \times 100\%$$

D% = percentage of burn healing

d₀ = wound diameter on day 1

dx = wound diameter on day 14

The percentage of burn wound healing was then statistically analyzed.

Results

The pH test results showed that all formulas were in the 4.5-6.5 range which did not cause skin irritation. Carbopol in aqueous solution has a pH of 2.5-4 so triethanolamine is needed as a buffer.¹⁰ The following are the results of measuring the pH of the ointment hydrogel:

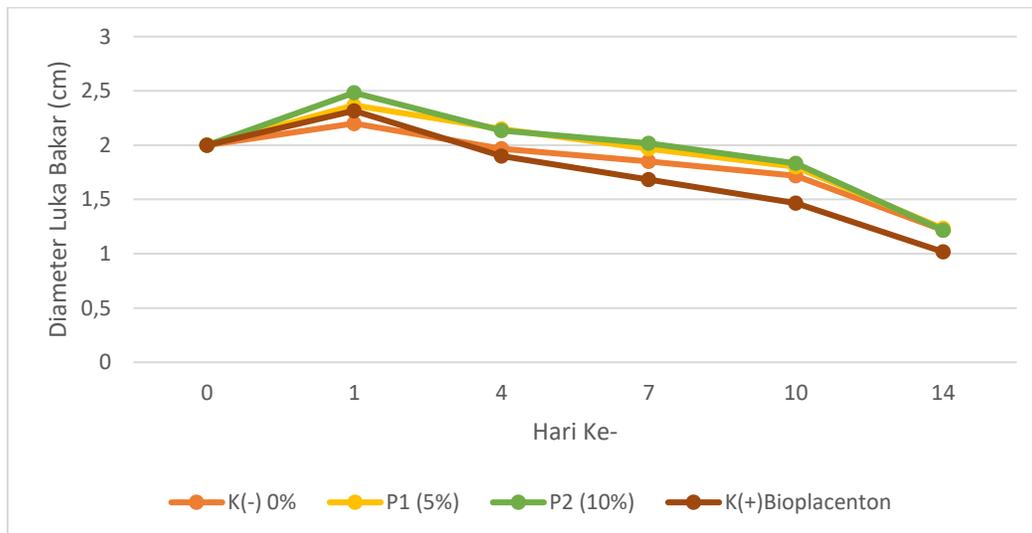
Table 2. pH of Hydrogel Ointment

No	Hydrogel Ointment	pH
1	Hydrogel Ointment Without <i>Sargassum sp</i> extract	6
2	Hydrogel Ointment <i>Sargassum sp</i> extract 5%	5
3	Hydrogel Ointment <i>Sargassum sp</i> extract 10%	5
4	Bioplacenton	6

The diameter of the burn was measured for 14 days, the measurement of the diameter of the wound was analyzed to determine the progress of wound closure. The results of the calculation of the average wound diameter with an interval of 4 days for each group are made in the form of tables and figures below:

Table 3. Mean of Diameter (cm) of Burns Wound by Group

Groups	Day 0	Day 1	Day 4	Day 7	Day 10	Day 14
K(-) 0%	2,000	2.200	1.967	1.850	1.717	1.217
P1 (5%)	2,000	2.367	2.150	1.967	1.800	1.233
P2 (10%)	2,000	2.483	2.133	2.017	1.833	1.217
K(+) Bioplacenton	2,000	2.317	1.900	1.683	1.467	1.017

**Figure 1.** Mean of Diameter (cm) of Burns Wound by Group Day 0 – Day 14

The data from the measurement of the diameter of the burn wound were analyzed to produce a calculation of the percentage of burn healing. The percentage of burn wound healing was then analyzed statistically

one way ANOVA. Before the one-way ANOVA statistical analysis is carried out, the data needs to be tested for normality and homogeneity. The results of the normality and homogeneity test showed p value >

0.05 so that the data were normally distributed and homogeneous. The results of one-way ANOVA statistical analysis p value 0.627 (> 0.05) which

showed no significant difference between the treatment groups. The results of the average percentage of burn healing are in table 4 and figure 2.

Table 4. The Mean of Percentage (%) of Burn Healing

Group	Average	
K(-) 0%	44.85 + 2.46	<i>P Value 0.627</i>
P1 (5%)	49.41 + 9.21	
P2 (10%)	50.31 + 3.64	
K(+)Bioplacenton	55.75 + 5.57	

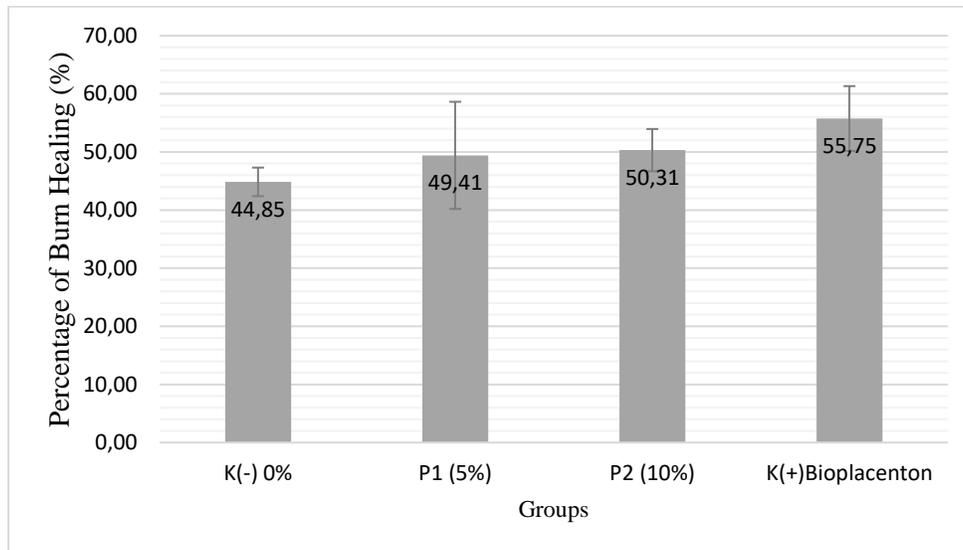


Figure 2. The Mean of Percentage (%) of Burn Healing

There were no significant differences in all treatment groups, but from the average percentage of burn healing, the bioplacenton control group showed the most optimal results. And in the negative control group, the ointment hydrogel without *Sargassum sp* extract showed the lowest percentage of burn healing.

Discussion

The results of measuring the pH of the hydrogel ointment, whether containing *Sargassum sp* extract or not, resulted in a gel base pH value of 5-6.

For plain ointment hydrogel without extract and bioplacenton the resulting pH is 6, while the *Sargassum sp* extract ointment hydrogel produced pH 5. Increasing the concentration of the extract in this study resulted in an increase in the pH of the ointment hydrogel, this indicates the acidity of the extract. In this study, the ointment hydrogel produced a pH range that was in accordance with the research of Prasongko et al, 2020 and Ardianti et al, 2018 where the pH value of an ointment hydrogel must match the skin pH, which is 4.5-6.5.⁹ The pH value that is too

acidic can cause skin irritation and too alkaline can cause scaly skin. While the pH of the hydrogel ointment with a range of 4.5-6.5 does not cause skin irritation.¹¹

The results of statistical analysis showed that there was no significant difference between the treatment groups, this indicates that the ointment hydrogel *Sargassum sp* extract had no significant effect on the control group, both the negative control group (without *Sargassum sp* extract) and the positive control group (bioplacenton). From the results of statistical analysis it is possible that the healing process of burns is relatively the same. This is possible because of the alleged human error during the process of making burns so that the burns formed are not second degree burns. Second-degree burns are characterized by damage to the epidermis and part of the superficial dermis, partial necrosis of tissue cells, and swelling and degeneration of some cells in the superficial dermis.¹²

In Figure 1 the results of measuring the diameter of burns from day to day in all treatment groups show that the burns are shrinking and undergoing a healing process. And in Figure 2 the results of the analysis of the calculation of the percentage of burn healing indicate the percentage of healing in each treatment group. In the negative control group with ointment hydrogel without *Sargassum sp* extract the percentage of healing that occurred was 44.85%, in the treatment group 1 with ointment hydrogel with *Sargassum sp* extract 5% the healing percentage was 49.4%. In treatment group 2 with ointment hydrogel with *Sargassum sp* extract 10% the percentage of healing was 50.31% and in the

positive control group with *bioplacenton* the percentage of burn healing was 55.75%. The percentage of burn healing in the negative control group without *Sargassum sp* extract had the lowest percentage of healing when compared to the percentage of burn healing in the treatment group 1 and treatment 2 with hydrogel ointment containing *Sargassum sp* extract.

Sargassum sp is one of the seaweed plants that has a lot of content and nutrients. The active ingredients of *Sargassum sp* include alkaloids, triterpenoids, steroids, saponins, phenols, flavonoids and quinones⁸ Wound healing process can occur due to secondary metabolites, namely flavonoids and saponins. In the wound healing process, saponins play a role in repairing damaged endothelial cells (angiogenesis) in the wound so that the supply of oxygen and nutrients becomes more optimal. In addition, saponins also function as antibacterial so that they can reduce the risk of wounds being contaminated by bacteria and can act as antioxidants that can minimize free radical levels in wounds so that the process of wound proliferation and contraction takes place more quickly. Saponins also promote epidermal cell proliferation and keratin cell migration.¹³ Finally, wound closure takes place more quickly.

One of the major causes of delayed healing is the persistence of inflammation or an inadequate angiogenic response. Flavonoids are substances that are believed to be anti-inflammatory and antioxidants.¹⁴ Flavonoids can block the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, this causes the

synthesis of inflammatory mediators such as prostaglandins, thromboxane is inhibited so that it can reduce inflammation.²

The flavonoid content in *Sargassum sp* extract can inhibit cyclooxygenase and lipoxygenase enzymes in the inflammatory cascade reaction so that it can reduce the production of prostaglandins and leukotrienes. The decrease in prostaglandins as proinflammatory mediators can limit inflammatory cells in the wound area. Suppression of prostaglandins as inflammatory mediators can cause reduced pain and swelling, and reduce vasodilation of blood vessels and local blood flow, so that the inflammatory reaction will last for a shorter time than the proliferation process can occur immediately.¹⁵

In addition to the active ingredients of secondary metabolites, *Sargassum sp* also contains other ingredients, namely zinc, calcium, iron, vitamin A and vitamin C which are known to accelerate the inflammatory phase. Vitamin A and Vitamin C contained in *Sargassum sp* can play a role in increasing the migration of neutrophils and macrophages to the wound area so that phagocytosis activation becomes optimal. Vitamins A and C also increase the synthesis of collagen and help in the differentiation of epithelial cells. Calcium contained in *Sargassum sp* can accelerate re-epithelialization by increasing the proliferation of keratinocytes. The iron contained in *Sargassum sp* can affect the process of cell growth and tissue maintenance, serves as a cofactor for collagen synthesis. Zinc contained in *Sargassum sp* also plays a role in wound healing, besides that it is also able to increase immunity.⁷

In Figure 2 the most optimal percentage of healing is in the positive control group with *bioplacenton*. This shows that *bioplacenton* is still more effective for healing burns than hydrogel ointment with and without *Sargassum sp* extract although the results of statistical analysis are not significantly different. So it is also necessary to evaluate the process of making ointment hydrogel with and without *Sargassum sp* extract.

Conclusion

In this study, the extract of seaweed (*Sargassum sp*) which was formed in the hydrogel ointment had no effect on the healing of burns.

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Conflict of Interest

The author stated there is no conflict of interest

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