Effect of extract gel of Sargassum sp. against histopathological of the number of fibroblast cells in the burn healing process

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ABSTRACT

**Introduction:** Burns are injuries caused by heat and cause damage to the skin. Burns are characterized by red, peeling skin. Burns must be treated properly so that wound healing can close completely. In Indonesia, seaweed is a promising commodity. From 5.2 million wet tons in 2011 to 9.2 million tons in 2013, seaweed output increased by 76.4 percent in Indonesia. This study aimed to study the histopathological picture of burn tissue given Sargassum sp.

**Methods:** An experimental study design with a randomized post-test-only control group design was used for this study. White mice (Mus musculus), the study’s population, made up this group. The study was divided into 4 treatment groups. The results of the calculation of the number of fibroblast cells were then evaluated using statistical tests to determine differences between groups. Data analysis was carried out using SPSS ver. 25 with a One-Way ANOVA test.

**Results:** The mean result of fibroblast cell count in group 1 (plain gel) was 36.14 PLP, group 2 (Sargassum sp. 5%) was 51.28 PLP, group 3 (Sargassum sp. 10%) was 53.36 PLP and group 4 (Sargassum sp. bioplacenton) 20.05 PLP. The results of the statistical test showed that there was a significant difference between groups with a p-value of 0.03 (<0.05).

**Conclusion:** The results of the histology show that there is a potential content of Sargassum sp. to affect wound healing.

**Keywords:** Histopathology, Sargassum sp., burns.


INTRODUCTION

Burns are a global and important problem because the effects of morbidity and complications caused by burns are very serious. Burns are heat-related wounds that harm the skin. Skin that is reddish and peeling is a sign of a burn. One of the injuries that frequently occurs in society and becomes a major issue is burns. According to World Health Organization (WHO) statistics from 2004, there were 11 million burn victims worldwide who needed medical attention. The American Burn Association (ABA) estimates that 450,000 burns occur in the United States each year. In 2013, there were 0.7% more burns in Indonesia than there were in 2008, a 1.5% decline.1

Similar to other types of wounds, the healing process for burns is divided into three stages: the inflammatory stage, which is marked by edema, fluid extravasation, and vasodilation; the proliferative stage, which is marked by revascularization and wound closure with the generation of keratinocytes. Revascularization, which increases collagen production by supplying fibroblasts with glucose and amino acids. The remodeling phase is characterized by wound maturation through the production of collagen and elastin by fibroblast cells. Collagen creation takes place during this phase.2-3

Indonesian seaweed production has expanded dramatically, rising by 76.4 percent from 5.2 million wet tons in 2011 to 9.2 million tons in 2013. Seaweed is a promising commodity in Indonesia. Flavonoids, saponins, tannins, and albumin are examples of bioactive substances found in seaweed that are beneficial as antibacterial, anti-obesity, cholesterol-lowering, anti-inflammatory, immunostimulant, and antioxidant agents.4 The four kinds of seaweed—green (Chlorophyta), red (Rhodophyta), brown (Phaeophyta), and blonde (Chrysophyta)—are dependent on the amount of pigment they contain. Various forms of seaweed, such as agar, piccolo, carrageenan, and alginate, are used in a variety of industries. In the medical field, seaweed is used to quicken the healing of wounds.5 Sargassum sp. contains alkaloids, triterpenoids, steroids, saponins, phenols, flavonoids, and quinones among its active components. Flavonoids and saponins are examples of secondary metabolites that can contribute to the healing of wounds. Saponins have a function in the wound healing process by rebuilding injured endothelial cells (angiogenesis) in the wound so that the delivery of oxygen and nutrients is improved.6-8

Therefore, there is a need for further studies on the histopathological and number of fibroblasts cell of burn tissue given Sargassum sp. This study aimed to study the histopathological picture of burn tissue given Sargassum sp.
METHODS

Study Design
In this study, measurements were only taken at the conclusion of the research utilizing an experimental study design with a randomized post-test-only control group. This was predicated on the presumption that the unit attributes were constant across populations. White mice (*Mus musculus*), the study’s population, made up this group. Males between the ages of 2-3 months, 20-30 grams in weight, healthy, defined as having active movements, clean and smooth fur, clear eyes, no abnormal discharge from the eyes, ears, or anus, no defects, and no weight loss of up to 10% during the acclimatization period were the inclusion criteria for each group, from which samples were taken.

Data collection procedures
The study was divided into 4 treatment groups, treatment group 1 (P1) was a group given burn treatment and hydrogel plain ointment without *Sargassum sp.* (0%) extract per day. Treatment group 2 (P2) is a group given burn treatment and hydrogel ointment containing *Sargassum sp.* 5% extract per day. Treatment group 3 (P3) was a group given burn treatment and hydrogel ointment containing *Sargassum sp.* 10% extract per day and treatment group 4 (P4) was a team given burning treatment and hydrogel bioplacenton ointments per day.

*Sargassum sp.* was obtained from Lampung Province and dried in the sun. *Sargassum sp.* was dried and then extracted by maceration using 90% ethanol. Then the extract of *Sargassum sp.* was added to the plain hydrogel with a concentration of 5% and 10%. The number of samples required for this study was calculated using the Federer formula with 6 mice in each group. Hydrogel ointment was given to the whole group for 14 days after the burn. after the 14th day, the entire body was scavenged in euthanasia and the skin tissue subjected to the burns was taken. Skin tissues were scavenged in 10% formalin and made in histopathological preparations to then evaluate the number of fibroblast cells. The results of the calculation of the number of fibroblast cells were then evaluated using statistical tests to determine differences between groups.

Data analysis
The fibroblast count data were analyzed statistically, to find out there was a significant difference, the statistical test used was the One-Way ANOVA statistical test. By the requirements of the previous One-Way ANOVA, the normality test of the Saphiro-Wilk test was carried out. Analysis was carried out using SPSS ver. 25. Results were considered significant if p-value≤0.05.

RESULTS
The results of the normality test of the data on the number of fibroblast cells showed a p-value>0.05 so the data could be said to be normal. Furthermore, the homogeneity test was carried out where the results of Levene’s homogeneity test also showed a p-value>0.05. From the results of the normality test and homogeneity test, the One-Way ANOVA statistical test can be carried out. The results of the One-Way ANOVA test showed p-value=0.003 where the result was less than 0.05, so it can be said that the results have a significant difference.

![Figure 1. The average number of fibroblast cells per group.](image1)

![Figure 2.](image2)
DISCUSSION

The inflammatory phase, the proliferation phase, and the remodeling phase are the three stages of the healing process for burns. During the inflammatory phase, neutrophils move to the site of the wound to phagocytize bacteria and already present foreign objects. Granulated tissue development, re-epithelization, and angiogenesis take place during the proliferation phase. The results of the average number of fibroblasts in the group containing extracts of Sargassum sp. had an average number of fibroblasts higher than the average number of fibroblasts in the group without Sargassum sp. In the group with 10% Sargassum sp. content, there was an increase in the number of fibroblasts from the group with 5% Sargassum sp. content. This indicates the possibility that the group containing Sargassum sp. has the opportunity for faster tissue repair as indicated by the large number of fibroblasts.

In the present study, 5% and 10% Sargassum sp. were combined with hydrogel-shaped wound wraps. A relatively recent class of wound packing materials is hydrogel. High sensitivity to the physiological environment, the water content in soft tissues, and appropriate flexibility are only a few of the unique qualities of hydrogel. Patients recovering from burn injuries benefit greatly from the use of hydrogel-shaped wound wraps. This hydrogel’s application has a multipurpose impact because it can be applied to practically all body parts, serves as a covering and cooling agent, and may take heat away from a wound. Additionally, agents with anesthetic, nutritional, or anti-inflammatory properties are added to hydrogel-shaped wound wrapers. The impact of brown algae extract Sargassum sp. on the healing of traumatic ulcers has been researched by Annisa et al., 2018, among other studies on the benefits of seaweed for wound healing, and a study by Aprinaldi, 2020 regarding the effectiveness test of red seaweed on wound healing. In this previous study, the effectiveness of Sargassum sp. seaweed was investigated in the form of an extract gel for the healing process of burns through the diameter of the burn. There has been no microscopic study of the effectiveness of Sargassum sp. against burns.

On days 4 through 21, the proliferation phase typically takes place. In this stage, granulated tissue will replace the transient wound matrix created during hemostasis. Granulated tissue is made up of numerous fibroblasts, granulocytes, macrophages, blood vessels, and collagen fibers that help to partially restore the skin’s structure and function. Granulated tissue is formed by fibroblasts, which migrate to the wound mostly from the nearby dermis in response to cytokines and growth factors like PDGF, (TGF)-β, and β-FGF. If the condition of the injury lasts for a long time, fibroblasts on the wound can also come from fibrocytes, which are a group of mesenchymal progenitor cells that originate from the bone marrow. Circulating fibrocytes go to the region of the skin that has been wounded and aid in healing by supplying a portion of the fibroblasts in the wound, as well as by releasing cytokines, chemokines, and growth factors, serving as antigen suppliers, and promoting angiogenesis. After moving to the temporary wound matrix, fibroblasts multiply and produce proteinases like the metallopeptinase matrix (MMPs), which degrade the temporal matrix. Meanwhile, they deposit collagen and other extracellular matrix components (ECMs), like proteoglycans, hyaluronic acid, glycosaminoglycan, and fibronectin, to form granulated tissue, which fills the wound gap and acts as a support for cell adherence. This study still has several limitation such as still consisted of unknown compounding variables that were not assessed yet in this study.

CONCLUSION

Histopathological description showed the average number of fibroblasts in the group containing Sargassum sp. extract was higher than the average number of fibroblasts without extract.

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The authors declare no funding in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

ETHICAL STATEMENT

The Ethical Committee of Nursing and Midwifery Faculty, Universitas Nahdlatul Ulama, Indonesia, has certified that this research is ethical with No: 298/EC/KEPK/UNUSA/2021.

AUTHOR CONTRIBUTION

Lysa Veterini conducted the measurements, Tri Wahyuni Bintarti was involved in the planning and supervision of the project, Tri Deviasari Wulan processed the experimental data, carried out the analysis, wrote the text, and created the figures. The statistical analysis and XYZ calculations were carried out by the entire crew. The entire team contributed to the interpretation of the findings and worked on the report. The findings were discussed and the text was reviewed by all authors.

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