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Potential of Sodium Alginate in *Sargassum* sp. in Lotion Preparation to Treat Incision Wound in Mice

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Abstract. *Sargassum* sp. is a known genus of brown seaweed whose dominant component is alginic acid or alginate. Various studies found that alginate having proinflammatory activity, so it may have potential to improve the wound healing process. This study aimed to obtain sodium alginate extract and to know the potency of the sodium alginate extract in incision wound treatment in mice abdomen. Lotion application was performed once a day in a span of five consecutive days. Observation on the wound was carried out to obtain data on blood coagulation, tissue reparation, and fibroblast development rate on the wound using histology. The results showed that the average yield from the *Sargassum* sp. extraction process was 31.3%. Quality control of the prepared lotion included organoleptic test; light brown coloration, lotion specific odor, semi-solid texture. The acidity of the lotion was measured as 6.61 with 6.731 cp of viscosity. The lotion adhesiveness test showed 0.21 seconds and the lotion was shown to be oil-in-water. Application of the lotion on incision wounds made in the abdomen of rats showed blood coagulation on the first day and onset of wound reparation process on the second day. Wound observation by the third day showed that the inflicted area had undergone a near-complete reparation. The study also showed that on the fifth day of reparation *Sargassum* sp. extract lotion treatment group showed fibroblast formation, as opposed to the non-extract lotion treatment group. Previous study use alginates as wound dressing material, but in this study alginate used as active ingredient in wound healing treatment. Therefore, this study concludes that sodium alginate in *Sargassum* sp. seaweed has a potential application in the field of medicine.

Key words: fibroblast, histology, incision wound, skin, sodium alginate

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INTRODUCTION

A wound is a situation in which body tissue is damaged (broken) or lost due to cuts, blunt force, changes in temperature, chemical substances, explosion, animal bite, etc (Jones & Bartlett, 2011). Improperly handled, the wound will fester, become infected, and may leave marks. Infection may cause continuous pain and burns, morbidity, an increase in medical bills, and even death (Dai, et al., 2011).

Disinfectants such as Rivanol® (Ethacridine Lactate) and Betadine® (povidone-iodine) may cause undesirable side effects such as irritation which can hinder wound reparation and even worsen the infection (Rachmawati, 2014). On the other hand 70% Alcohol may damage healthy skin tissue in the periphery of the wound as it is the nature of the disinfectant to damage cell walls and denature protein (Jung et al., 2011).

Seaweed is a natural marine bioresource with numerous proven and potential applications as a bioactive compound material (Liu et al., 2013). Based on its pigments, seaweed falls into three categories; green (Chlorophyceae), red (Rhodophyceae), and brown seaweed (Phaeophyceae) (Osorio et al., 2020). *Sargassum* sp. is a known genus of brown seaweed whose dominant component is alginic acid or alginate. Alginate is a linear polysaccharide polymer that consists of α-L-guluronate and β-D-manuronate (Yudiati et al., 2016). Various studies found that alginate exhibits properties for immuno-stimulant activities in grouper fish (Harikrishnan et al., 2011) and shrimps (Chung et al., 2011), anti-tumor (Cong et al., 2014), antioxidant (Sellimi et al., 2015), as well as a proinflammatory agent (Kézia et al., 2013). In addition, alginate can also be used as a treatment in primary wound dressing (Devi et al., 2012). The properties of alginate found in those studies show its potential in expediting wound reparation.

Based on the findings of previous studies, a study of the healing properties of alginate in lotion preparation is needed. This study aimed to determine the comparative results of lotion quality between sodium alginate extract of *Sargassum* sp. and non-extract lotion. Furthermore, this research also sought to determine the effect of sodium alginate lotion from the extract of *Sargassum* sp. on wound reparation rate using histological observation. Therefore, sodium alginate lotion was expected to be a viable alternative as a natural wound recovery medicine that can reduce the wound recovery time without damaging other health cells around the wound.

METHODS

The material used in this study was the lotion preparation of sodium alginate extracted from *Sargassum* sp. Subjects for the experiment were 1-month-old female lab mice (*Mus musculus*) from LPPT Gadjah Mada University. Live subject experimentation was approved by Commission for Ethical Health Research Diponegoro University by Ethical Clearance No. 10/EC/H/FK-RSDK/III/2018.

Sodium alginate extraction

Prior to extraction, clean and fresh samples of *Sargassum* sp. were air-dried and cut into pieces. Fifty mM EDTA and 5% Na2CO3 were added with HCl until the pH reached 8.4. *Sargassum* sp. were soaked in the solution for 24 hours, with periodical stirring. The solution was sieved to obtain the pellets and the resulting product was added with 0.13 KCl, mixed with 96% of cold ethanol, in a 1:1 volume ratio. Both solutions were mixed in a centrifuge mixer at 3500 rpm for 5 minutes. Pellets were taken and put into the oven at 60˚C for approximately 24 hours. The dehydrated alginate was then ground into powder (Yudiati & Isnansetyo, 2017).

The percentage of alginate yield can be calculated by dividing the mass of alginate extract with the mass of extracted dehydrated seaweed. In the formula, the calculation of alginate yield is written as follows:

Alginate Yield (%)=(Alginate mass)/(Seaweed mass)×100%

Lotion preparation

NaEDTA was diluted in distilled water and designated as Part A. Carbopol was diluted in hot water and mixed with the part A. Part B material was prepared by diluting propylene glycol, glycerin, nipagin, and nipasol in hot water while heated up to 65˚C until all materials were thoroughly mixed. Part A and Part B materials were then mixed. Liquid paraffin, stearic acid, glycerol monostearate, cetyl alcohol, and distilled water were mixed and heated up to 65˚C and the solution was designated as the Part C. Mixture of Part A and Part B were added into Part C, after which the mixture was put into homogenization process. Triethanolamine (TEA) was diluted in distilled water, which was designated as the Part D. Part D was added into the mixture of Part A, Part B, and Part C, and was then homogenized using a stamper, with the subsequent addition of distilled water until the ideal base lotion was obtained.

Lotion preparation quality test

The quality of lotion in this study was tested based on Olesiuk et al. (2013) methods with some modifications for its organoleptic (texture, color, and smell), acidity, viscosity, spread rate, adhesiveness, also type of lotions. The organoleptic test employed in this study covered texture, color, and smell. The pH of the lotion was measured using a pH meter, whereas the lotion viscosity was measured with a Brookfield Viscometer. Lotion spread rate was determined by measuring the constant diameter of lotion placed on a 10 x 10 cm square glass plate after being pressed by weights of 100, 150, 200, and 250 grams each for 1 minute. Lotion adhesiveness was determined by measuring the time of separation of two glass objects with the lotion in between and pressed with 50 grams of weight.

Shaving, wounding, and lotion administration treatment

The abdominal hair of eight female lab mice were shaved off. On the shaved part of the abdomen of each mice, ±0,5 cm wound was made using a disinfected scalpel. Alginate extract and non-extract lotion were then applied to the wound, every day for five consecutive days. Observation of all subjects was made on their blood agglutination rate and tissue reparation.

Histology

Histology preparation was made in accordance with Luna (1968) and Culling and Dun (1974) with several modifications. Skin samples were fixated with Buffer Neutral Formalin (BNF) 10% solution, which were then dehydrated using alcohol 70%. Skin samples were then cleaned with a session of each solution consecutively, starting with formalin 10% I, formalin 10% II, and formalin 10% III. After cleaning, samples were dehydrated using alcohol 70%, alcohol 96%, absolute alcohol I, absolute alcohol II, and absolute alcohol III. Samples were then soaked in xylol I, xylol II, xylol III, liquid paraffin I, and liquid paraffin II solution. Finally, the skin samples were put into a blocking process using liquid paraffin until frozen, after which all samples were cut using the microtomic knife.

The next stage in making skin sample preparation was staining using haematoxylin eosin (HE) stain. The pigmentation employed in this study was Harris-haematoxylin eosin staining. The samples were soaked three times in xylol of 5 minutes each, followed by two times absolute alcohol. Afterward, samples were soaked in distilled water twice. After that, samples were soaked with acid alcohol 10%, continued with haematoxylin and eosin soaking. Stained samples were soaked in alcohol 96%, followed by soaking in absolute alcohol twice. Lastly, the samples were soaked in xylol twice.

Samples' histological observation

Samples in this study were observed using a light microscope (Leica, Germany) with 40x10 and 100x10 magnification factors. Based on Suwiti (2010), observed variables included histological development of wound by identification of fibroblast and inflamed cells.

RESULTS AND DISCUSSION

Alginate yield percentage

Data from sodium alginate extraction from *Sargassum* sp. in this study showed a yield of approximately 26.6 – 37.3%. The highest yield was found in the first cycle, 37.3%. The lowest yield was measured on the third cycle, 26.6%. Data of alginate yield are presented in Table 1 below.

Figure 1. The morphology of *Sargassum* sp.

Table 1. Sodium Alginate Yield of *Sargassum* sp.

Seaweed mass (gr)	Alginate mass (gr)	Alginate Yield (%)	Average Yield (%)
5	1.86	37.3	31.3 ± 5.467
5	1.50	30	
5	1.33	26.6	

Lotion Preparation Quality

Lotion preparation quality test in this study consists of organoleptic tests (texture, color, smell), adhesiveness test, pH test, viscosity test, and lotion type test. The various test conducted in this study serves to ensure that the lotion is suitable and do not trigger allergies for topical application (Megantara et al., 2017). The results of the quality test in this study are presented in Table 2 – Table 7 and Figure 1.

Organoleptic test

Organoleptic test on the lotion preparations, both with and without alginate extract, are as follows:

Table 2. Organoleptic Test Results of Lotion Preparations

Specifications	Alginate 0.1% Lotion	Lotion (without Alginate)
Texture	Semi-solid	Semi-solid
Color	Light brown	Off-White
Smell	Lotion Specific	Lotion Specific

Viscosity Test

The viscosity of the lotion preparations, both with and without alginate extract, in this study was measured at 6.731 - 9.622 cP. The viscosity test results of both lotion preparations are presented in Table 3 below.

Table 3. Viscosity Test Results of Lotion Preparations

Sample	Viscosity (cP)
Alginate 0.1% Lotion	6.731
Lotion (without alginate)	9.622

Note: cP = centipoise

Adhesiveness test

Test results showed that the viscosity of the lotion preparations in this study ranges between 0.15-0.21 seconds. Sodium alginate lotion showed more viscosity, 0.21 seconds, compared to non-alginate lotion preparation. The results are presented in Table 4 below.

Table 4. Viscosity Test Result of Lotion Preparations

Sample	Time (seconds)
Alginate 0.1% Lotion	0.21
Lotion (without alginate)	0.15

pH test

The acidity test of the lotion preparations obtained the value of 6.61 – 6.91. The test found that alginate lotion preparations had lower acidity of 6.61 as opposed to the non-extract lotion. The results of pH tests are presented in Table 5 below.

Table 5. Acidity (pH) Test Results of Lotion Preparations.

Sample	pH
Alginate 0,1% Lotion	6.61
Lotion (without alginate)	6.91

Lotion type test

Lotion type tests of both preparations were conducted using light microscopy and produced similar results. The resulting microscopy image is presented in Figure 6. Based on the results, image a is identified as emulsion type, with stable emulsion

which did not break on contact with water. Image b showed lotion stained with methylene blue color.

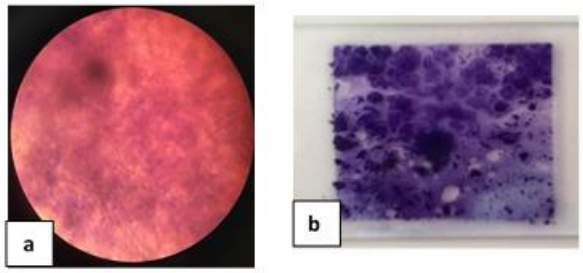

Figure 2. Micrograph identification of lotion type (a) dilution method using water (b) staining method; lotion type was identified as oil-in-water.

Methylene blue is a water-soluble stain and in image b it is shown to be diluted. Therefore, the dilution phase in the lotion is water.

Wound tissue observation

Direct observation on mice wound tissue was performed in 4 consecutive days after the subjects were cut with a sterile scalpel. The observation included blood coagulation and visual tissue reparation, which results are presented in Table 6 and Table 7 below.

Table 6. Blood Coagulation on Subjects

Lotion Sample	Day 1	Day 2	Day 3	Day 4
Sodium alginate	√	√	√	√
Without extract	√	√	√	√

Note: × = no blood coagulation; √ = blood coagulation

Table 7. Tissue Reparation on Mice

Lotion Sample	Day 1	Day 2	Day 3	Day 4
Sodium alginate	×	√	√√	√√
Without extract	×	×	√	√√

Note: × = Open wound; √= partial reparation, √√= complete reparation

Blood coagulation on both treatments took place immediately after the application of lotion. On the other hand, wound tissue reparation on both treatments showed a marked difference. The alginate lotion treatment group showed tissue reparation on Day 2, whereas the non-extract lotion group started showing the same activity on Day 3. Therefore, sodium alginate extract lotion was found to have better effect on wound recovery.

Once direct observation was concluded, a histological observation was performed. The following images are the results of histological observation on the subjects which had been wounded and underwent two different lotion preparation treatments for 5 consecutive days. The formation of fibroblast on wound tissue is presented in Table 8.

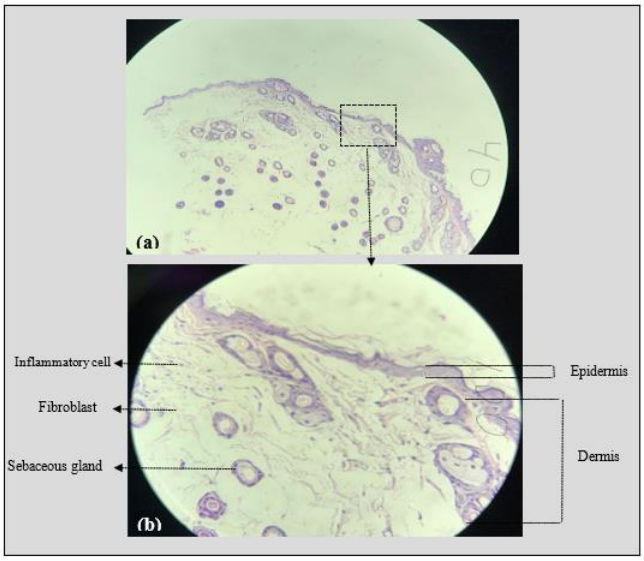

Figure 3. Micrograph showing tissue on the test subjects with alginate lotion treatment. Magnification factor 400X (a) and 1000X (b).

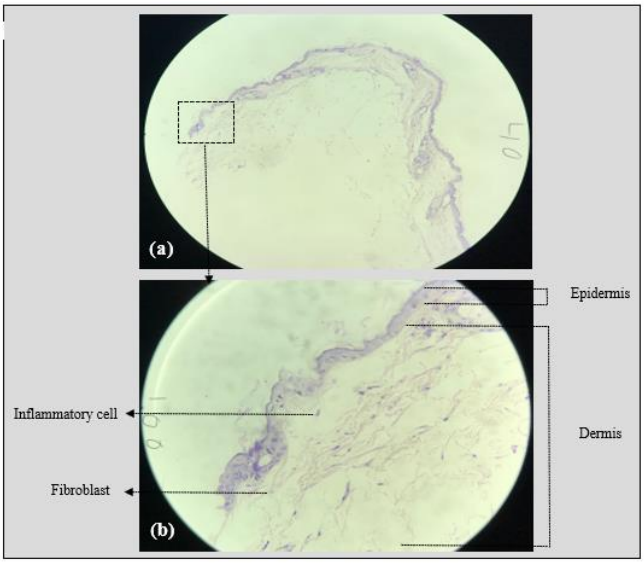

Figure 4. Micrograph showing tissue on the test subjects with non-extract lotion treatment. Magnification factor 400X (a) and 1000X (b).

Haematoxylin-Eosin stained histology images would show fibroblast as fine threads in pink coloration.

Table 8. Fibroblast Formation Histology on Lotion Preparation Treatments

Treatment	Mag. Factor.	
	400X	1000X
Sodium alginate lotion	Little fibroblast	Good, uneven fibroblast
Non-extract lotion	No fibroblast	Little fibroblast

Histological observation on the subject treated with alginate lotion on 400x magnification factor showed the existence of little fibroblast on the immediate dermis surrounding the wound. On 1000x magnification factor, more fibroblast was clarified albeit in an uneven structure. Analysis on the subject treated with non-extract lotion on 400x magnification found no fibroblast formation, although on 1000x little fibroblast was found.

Both treatments resulted in the difference in the rate at which fibroblast formation took place, sodium alginate treatment shown to better encourage the formation of fibroblast compared to the non-extract lotion treatment.

Lotion preparation quality testing is performed to determine whether a product is comfortable to use and does not have the potential to trigger allergic reactions upon topical application (Megantara et al., 2017). Organoleptic observation of the lotions in this study found that the products were semi-solid in texture, with lotion specific smell. The viscosity of sodium alginate lotion was measured at 6.731 cP, lower compared to the non-extract lotion, at 9.622 cP. The viscosity of both lotion preparations is of good quality since the maximum limit of viscosity for lotions is at 30.000 cP (Marchaban & Saifullah, 2014). However, it is interesting to note that the sodium alginate lotion showed lower viscosity compared to the non-extract lotion, since alginate is known to have properties of gelling agent (Draget & Catherine, 2011). Such properties stem from alginate's polymer chain length (Maleki et al., 2017). The adhesiveness test showed that sodium alginate lotion possessed greater adhesiveness, 0.21, than non-extract lotion, 0.15. Sodium in the lotion preparation is thought to contribute to the greater adhesiveness. Viscosity and adhesiveness affect the rate at which lotion is absorbed by the skin. Lotions with lower viscosity and adhesiveness have a higher absorbance rate, and in turn, leave no abnormal sensation in the skin immediately upon topical application.

Acidity for sodium alginate and non-extract lotion was measured at 6.61 and 6.91 respectively. The tolerance limit in pH for lotion ranges between 4.0 to 7.0, making both lotion preparations unlikely to trigger any allergic reaction due to excessive acidity or base (Megantara et al., 2017). Under microscopy, both lotion preparations showed properties of oil-in-water, because dilution test by adding water on lotion, as shown in Image 6 (a), did not result in texture breakdown. In other words, the water added into the lotion can integrate with the dispersing phase of the lotion. Lotion with oil-in-water properties has water as its dispersing phase, making the oil as the dispersed component. Image 6 (b) showed the emulsion-type test with staining to enhance results. The stain used in this study is methylene blue. The image shows that the stain is diluted in lotion preparations, thereby confirming the finding that water is the dispersing phase and oil is the dispersed phase since methylene blue can only be diluted in water.

Direct observation of wound tissue was conducted on the parameters of blood coagulation and tissue reparation. Histological analysis on wound tissue was also performed on the variable of fibroblast growth. Fibroblast is a building block of granulation tissue in addition to epithelial cell and keratinocyte. Fibroblast forms collagen fibers in the proliferation process during the tissue reparation (Koehler et al., 2018).

Blood coagulation rate on the wound of subjects under both lotion preparation treatments did not show a marked difference. Both treatment produced immediate blood coagulation after topical application of lotion preparation. Blood coagulation in flesh wound took several minutes by thrombocyte aggregation and solidification of fibrin tissue (Koehler et al., 2018).

Tissue reparation observation found a marked difference between sodium alginate lotion treatment and non-extract treatment. The faster reparation was seen in subjects with sodium alginate treatment, of which onset was observed on day 2 of the treatment. Observation on the same day showed no tissue recovery activity in subjects with non-extract lotion. Tissue reparation only occurred on day 3 on the subjects with non-extract lotion treatment, whereas subjects with sodium alginate lotion treatment had achieved good tissue reparation by that time.

Fibroblast is an indicator in wound tissue reparation process. It is formed during the proliferation phase (Wosgrau et al., 2015). It will turn into collagen, an important building block for the skin (Chattopadhyay & Raines, 2014). Fibroblast which is used in forming collagen is called fibrocollagen. In skin micrography shown in Image 7 and Image 8, the fibroblast is seen as thin fibers with pink coloration under HE (Haematoxylin-Eosin) staining. In 400x magnification factor, the sample from the sodium alginate treatment showed little fibroblast formation. However, in 1000x magnification factors, more fibroblast could be seen, although it was unevenly spread. On the other hand, the sample from the non-

extract lotion treatment showed no fibroblast formation in 400x magnification factor, with little formation shown in 1000x magnification factor.

Alginate is known for its properties in inducting monocyte cells and macrophage stimulation which increases the production of pro-inflammatory cytokine. The mechanism of alginate when inducting monocyte cells and macrophage stimulation has not yet been determined, but it is postulated that alginate affects the stimulation of macrophage in the NF-kB cell line. Pro-inflammatory cytokines are divided into several types, namely IL-1β, IL-6, IL-12, dan TNF-α (Luo & Zheng, 2016).

Neutrophils are immunity cells which acts as the first line of defense following tissue damage. It acts as a natural antimicrobial agent which sterilizes the area surrounding the wound from microbe and foreign materials through phagocytosis. After sterilization has been completed, neutrophils undergo apoptosis which is then removed by the macrophage. The role of neutrophil in the process of wound tissue sterilization is assisted by Natural Killer T (NKT) cells. The cells produce cytokine and chemokine which increases fibro-proliferative aspect in wound tissue reparation (Stunova & Vistejnova, 2018). The increase in cytokine production will expedite the proliferation process, during which fibroblast is formed (Kim & Moudgil, 2017). Macrophage stimulation in producing cytokine is related to the ability of alginate to accelerate wound recovery (Thomas et al., 2000). Previous study use alginates as wound dressing material, but in this study alginate used as active ingredient in wound healing treatment. Therefore, sodium alginate lotion is a viable alternative as a natural wound recovery medicine that can reduce the wound recovery time without damaging other health cells around the wound.

CONCLUSION

The sodium alginate was extracted from *Sargassum* sp. with 31.3 ± 5.467 in average yield from seaweed's dried weight. The lotion sample with sodium alginate extract was found to provide and accelerate the wound recovery process. This medicinal property of alginate was indicated by the amount of fibroblast formation on the wound tissue after the use of lotion. Alginate can induce the inflammatory process, then the growth of the fibroblast can be faster. So, the sodium alginate lotion is a viable alternative as a natural wound recovery medicine.

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