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Original Research Article

Potential Effect of Red Algae Content on Fibroblasts and Collagen Type 3 in Oral Mucosa Wound Healing Diabetes-Induced *Rattus Norvegicus*

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ABSTRACT

Diabetes can lead to abnormalities in the mouth. Diabetes-related oral mucosal disorders are abnormalities associated with impaired salivary gland function and immune system modifications. Red algae are currently of concern because of their potency levels that affect the wound healing process, such as polysaccharides, amino acids, fatty acids, saponins, terpenoids, steroids, flavonoids, tannins, phenols, alkaloids, quinones, terpenoids. This study evaluated the effect of Red algae compositions from Kajhu, Aceh Besar, Indonesia on the oral mucosa wound healing process in diabetes-induced *Rattus norvegicus*. Red algae extract was produced by the maceration process with 70% ethanol 25L. Phytochemical and GCMS tests were carried out, to determine Red Algae content. Red algae gel was applied to the oral mucosa incision in diabetic *Rattus norvegicus* to investigate the wound healing process. The parameters studied included wound healing contraction, epithelialization, fibroblast counts, and collagen type 3. The composition of the red algae (*Gracilaria sp.*) from Kajhu was dominated (39.80%) Hexadecanoic acid. (9e)-9-octadecanoic acid, (11.34%). 1,2-Benzenedicarboxylic acid (6.7%), followed by Erythritol (6.49%), and Cholesterol (5,12%). The data was analyzed using the ANOVA test with significance ($p < 0.05$), post hoc test with Tukey HSD. The gel contains a 3% extract of *Glacilaria sp.* improves wound healing clinically and histologically. During the proliferative phase, the number of fibroblasts increased, the thickness of the epithelium increased, and it showed regular and cross-linked collagen.

***Keywords*:** Red Algae, Fibroblast, collagen type 3, Diabetes, *Rattus Norvegicus*

Introduction

Diabetes is defined as a metabolic disorder due to disorders of the pancreas in producing insulin or the use of insulin by the body which is not potent.[1] Between 2000 and 2016, there was a 5% increase in deaths from diabetes. The early death rate from diabetes increased in both periods in lower-income countries.[1] Patients with diabetes mellitus frequently develop chronic wounds as a result of the impairment of wound healing. Diabetes impairs healing because of a complex pathophysiology that combines vascular, neuropathic, immunological, and metabolic components. Hyperglycemia is related with stiffer blood vessels, which results in slower circulation and microvascular dysfunction, impairing tissue oxygenation performance. Diabetes-related blood vessel modifications explain why leukocytes migrate less into wounds, rendering them more susceptible to infection. Hyperglycemic conditions can decrease leukocyte function. Peripheral neuropathy can produce numbness and a decreased ability to perceive pain, which can contribute to the chronicization of wounds that are not diagnosed and treated promptly.[2]

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In diabetics, the ability to regenerate and repair is decreased due to reduced blood flow, decreased immune system ability, and decreased ability to produce growth factors that play a role in tissue healing.[3] In diabetes, insulin resistance will appear where the accumulated fat tissue will inhibit the work of insulin in the body and muscle tissues so that glucose cannot be transported to cells. [4] In the repair and regeneration during oral wound healing, the speed of healing time is important to prevent millions of microorganisms from interfering with the wound healing process, especially in diabetic oral mucosal wounds to prevent inhibition of healing which can lead to tissue death.[5]

Diabetes causes changes in the oral cavity. Disorders of the oral mucosa that are common in diabetics are changes associated with decreased salivary gland function and changes in the immune system. The parts of the oral cavity most often affected by diabetes are the periodontal mucosa and tongue mucosa.[3,6,7] Studies Rashmi et all showed a person with type 2 diabetes was discovered three people were diagnosed with lichen planus, ulceration, and candidiasis. Acute necrotizing gingivitis was observed in four patients, one of whom was a control. (1.33%), and three in diabetes (4.0%), 49 people were diagnosed with chronic generalised periodontitis and localised periodontitisAbscesses were observed in 20 individuals (5 (6.67%) in the control group and 15 (33.33%) in the diabetic group, while 39 patients had more than one condition, 32 (42.67%) of the diabetic group and 7 (9.33%) of the control group were diabetes.[8]

Previous studies have shown that red algae contain polysaccharides [9], protein/amino acids,[10] and fatty acids,[11,12] that play a role in accelerating wound healing. Previous research has revealed that Red algae have also been found to contain saponins, terpenoids,[13] steroids, flavonoids, tannins, phenols, glycosides alkaloids,[14] quinones, and terpenoids.[15] Secondary metabolites can act as anti-bacterial, anti-inflammatory, and

antioxidant by preventing reactive oxygen species.[16] which can increase wound closure, epithelialization, angiogenesis, and stability of collagen matrix against collagenolytic degradation, in wound healing.[17]
Fibroblasts are important cells that move from the perimeter of the wound to the area that is damaged, mending the wound. The physiological process of returning wounded tissue to its original position is known as wound healing. It is vital to ensure that the healing processes run smoothly.[18] Fibroblasts in the wound region are derived from blood circulation, whereas significant fibroblasts are derived from mesenchymal cells in the bone marrow. Fibroblasts play a vital role in wound healing, eliminating temporary ECM, influencing inflammation, re-epithelialization, and angiogenesis, and producing and regulating new ECM for tissue repair. Type 3 collagen is the first collagen present in the remodeling phase of wound healing. Collagen is important for cell adhesion, cell migration, tissue morphogenesis, and scaffolding, also tissue repair. Collagen plays a role in providing structural support to resident fibroblast cells, regulating local cell and inflammatory cell function. Type 3 collagen is rapidly formed, with a low initial matrix that functions as a defence against infections and fluid loss, which is followed by its destruction by proteases and redesign by fibroblasts into type 1 collagen, which has a much higher tensile strength.[19]
The purpose of this study is to identify the metabolite of *Gracilaria sp*. Aceh sea waters and its constituent substances are to be tested on diabetic experimental animals by clinically and histologically measuring the number of fibroblast cells, epithelization, and collagen type 3.

Material and Methods

Material
The material used is Red Algae, Ethanol 70%, magnesium, HCl, Dragendorff reagent, Mayer reagent, $FeCl_3$, H_2SO_4, acetate anhydride acid, iodine 0,01 N, distilled water, and nitric acid.

Instrumentation
The instrument used in this research are a Rotary evaporator, Gas Chromatography-Mass Spectrometry (an Agilent Technologies 7890, Santa Clara, CA, United States), Scalpel and blade No. 11, Periodontal probe (UNC-15) merk kohler, medizintechnik Germany, microscope Meiji Techno Co.Ltd. Japan, DP-12 digital camera, and Top View software.

Procedures
Red Algae Collection and identification (*Gracilaria sp*)
Red Algae (*Gracilaria sp.*) was obtained from kajhu Aceh Besar, 5°37'06.8"N 95°23'51.9"E (Figure 1) in December 2021 and identified by Fandri Sofiana Fastanti, MSi (Voucher number B-896/V/DI.05.07/3/2022). *Gracilaria sp.* is distinguished by its brown, dark olive, or dark red colour. Fronds are upright, cylindrical, and 25-30 cm tall. The long axis measures 1.5-2 mm in diameter, with lateral branches of varying diameters, typically 3-4 digits, alternating at irregular intervals in a unilateral pattern. Branches are typically constricted towards the base. The cortex is made up of 1-3 layers of pigmented cells. The outer cortical cells are radially elongated, ovoid, and range in size from 4.5-7 mm. The subcortical cells have dimensions ranging from 12-25 mm to 25-31 mm, while the medulla has huge isodiametric cells with diameters ranging from 150-360 mm. (Figure 2)

Red Algae extraction
Samples of *Gracilaria sp.* were extracted by maceration, and 10 kg of *Gracilaria sp.* was washed with water, ground, and dried at room temperature and for 8 days in the sun. The dried sample was ground into a powder and then weighed. A total of 1 kg of sample was soaked in 70% ethanol 25 L for 2 x 24 hours and filtered. The obtained filtrate was dried by evaporation at 40 ° C. Making use of a rotary evaporator to obtain an ethanol extract of red algae.

Phytochemical test
The *Glacilaria sp.* extracts were preliminary evaluated for the detection of flavonoids (Shinoda test), steroids and terpenoids (Liebermann–Buchard test), alkaloids (Dragendorff test), and sapponins (foam test), [20] tannin test (ferric chloride test) [21], phenolic test.[22]

GC-MS analysis
The components in the *Gracilaria sp.* ethanol extract were identified using GC-MS. It made use of gas chromatography. It has a 30 m SPB-50 column with a film thickness of 0.25 m and an interior diameter of 0.25 mm. 230 °C was established as the injection temperature and 250 °C served as the interface temperature. The ion source's temperature was set to 200 °C. The carrier gas employed was helium, and the flow rate was maintained at 1 ml/min. The temperature program that was used involved isothermal heating for 5 minutes at 70°C, followed by 5°C/minute temperature increases until the oven reached 310°C, and then heating for 1 minute at 310°C. Two scans per second were used to record the mass spectrum, which ranged from 50 to 600 m/z. The chromatogram and mass spectrum were examined using a mass lab application. Retention time and mass spectra were employed in a mass laboratory technique to automatically count the number of peaks of metabolites. The algorithm was obtained using Microsoft Excel. The National Center for Biotechnology Information (NCBI) database was used to identify compounds.[23]

Preparation of Red Algae gel
A base gel was made by combining 10 ml of hot distilled water with 0.125 g of Carbopol (Merck, Germany) and stirring the mixture with a pestle. The mixture was then mixed with 1.5 g of triethanolamine (TEA) (Merck, Germany) and 2 g of glycerol (Merck, Germany). 10 mL of distilled water, 0.125 g of hydroxypropylmethylcellulose (HPMC) (Merck, Germany), Nipagin (Merck, Germany), and Nipasol (Merck, Germany) were combined until homogenous in a second dairy dish. The second mortar mixture was poured into the first mortar and mixed until it was homogeneous. To make 20 g of red algal extract gel 3%, 0.6 g of red algae extract was mixed with the basic gel until the mixture was thoroughly mixed.[24]

Preparation and Induction of Diabetes using Streptozotocin in Experimental Rattus Norvegicus
The experimental animals were male *Rattus norvegicus*, average weight 200-250 grams, 10 weeks old, in good health with active movement characteristics, and thick fur (*Rattus norvegicus*). The Faculty of Syiah Kuala University's Veterinary Medicine Animal Laboratory provided experimental animals based on Federer's formula, a total of 15 rats for the positive control group, negative control group, and treatment group. Following a week of adaptation, the rats were given streptozotocin injections intraperitoneally at a dose of 35 mg/kg body weight in the rats. Rats are fed a typical meal for adult rats ad libitum. A week after the injection of streptozotocin, blood glucose measurements with gluco DR, when the rats were carried out from the tail vein (lateral vein), the results showed an average random blood sugar of 394.3 mg/dL.[25]

Figure 1: Coordinate Location Sub district Lamnga Kajhu (Aceh Besar) 5°37'06.8"N 95°23'51.9"E


Figure 2: Red Algae from Kajhu (private collection)

Making an incision wound on the gingiva of rats.
The rats were given a single intramuscular injection of 1-2 mg/kg xylazine hydrochloride and 10 mg/kg ketamine hydrochloride. A scalpel and a No. 11 blade were used to make an incision along the 5 mm depth to reach the bone.

Wound Healing observation
Observations of reduced wound diameter in rat lacerations were performed daily in each group using a periodontal probe (UNC-15). Wound healing occurs with a wound diameter of 0 mm or complete wound closure, which occurs during the proliferative phase when the re-epithelialization process is complete. After completion of the procedure, all rats were euthanized.

Histological Examination
Hematoxylin eosin staining
Wound sites on the oral mucosa of experimental animals were sliced along 10 x 5 mm and fixed in a 10% formaldehyde phosphate solution for 18-24 hours. Then washed for 15 minutes with distilled water. Furthermore trimming is used to cut tissue with a thickness of 2-3 mm. Immersion in graded alcohol at 70%, 80%, 90%, 95%, absolute I, and absolute II concentrations dehydrates tissue. Xylol was used for additional cleaning. Embedding or submerging the specimen inside the base mold in 70°C liquid paraffin to generate paraffin blocks. The paraffin is then sliced using a microtome to a thickness of 5 mm, and the tape is placed in a water bath at 40°-50°C until the tissue stretches and is taken using an object glass and dried with a glass warmer. Then the deparaffinization process with 3 xylol tubes, absolute alcohol I, alcohol absolute II, 96% alcohol, 90% alcohol, and 80% alcohol each for 2 minutes. The last stage is staining the specimen using hematoxylin and eosin. The slide is inserted into the hematoxylin for 5 minutes and then washed in water until clean, followed by dipping preparations in acid alcohol, then dipped once in water. Glass object was inserted into eosin for 3 minutes, 96% alcohol I, 96% alcohol II, absolute alcohol I, absolute alcohol II for 1 minute, and xylol for 2 minutes. Wait for the preparation to dry then the preparations were closed using a cover glass.

Immunohistochemistry staining collagen type 3 procedure.
Samples were taken from the tissue in the form of paraffin blocks. Each paraffin block was cut with a 4-micron microtome and placed on a glass object. Each paraffin block was cut, and sample pieces were stained by immunohistochemistry with collagen type 3 and DAB substrate. Started deparaffinization followed by rehydration then give distilled water. Inhibit endogenous peroxidase enzyme activity (dip), give distilled water and PBS, then give normal serum 1-2% then give PBS. Followed by incubation of primary antibody and PBS then Incubation with secondary antibody (biotinylated antibody). Incubate the ABC elite Kit and administer PBS. Visualized with 0.02% DAB in Triss Buffer plus 0.03% H2O2 and given PBS again. Rinse with water then distilled water and dehydration, clearing, and mounting.[26]
Use the Olympus BX41 microscope to observe and calculate the number of prepared fibroblasts and collagen. The magnification is 400x and each preparation has 5 fields of view. This observation is equipped with a DP-12 digital camera and uses Top View software. Quantitative analyses of collagen density were performed using the ImageJ software (https://imagej.nih.gov/ij/index.html).

Ethical approval
The experimental protocols were authorised by the Ethics Commission of the Faculty of Dentistry at Universitas Syiah Kuala. (271/KE/FKG/2021)

Data analysis
The data was analyzed using SPSS, and the hypothesis testing was carried out using the ANOVA test with significance ($p < 0.05$), post hoc test with Tukey HSD.

Result and Discussion

The wound is damage or disturbance to normal and functional anatomical structures, which can range from simple epithelial damage or can be deeper to subcutaneous tissue or other structures. Some dental procedures could cause tissue damage. When tissue damage occurs, the body will immediately activate a wound-healing response. Wound healing is a process of repairing wound tissue in a coordinated manner characterized by several stages, namely, hemostasis, inflammatory phase, cell proliferation phase, and remodeling phase. The purpose of this study was to examine the phytochemical constituents of *Glacilaria sp.* from Kajhu Aceh Besar to see how it affects the clinical and histological healing of oral mucosal wounds in streptozotocin–induced diabetic rats.
Diabetes is linked to decreased wound healing, putting patients at risk of persistent, incurable wounds. Chronic diabetes wounds have higher amounts of pro-inflammatory cytokines and proteases as well as decreased growth factor production, trapping them in a prolonged inflammatory state. Either directly by having an impact on systemic processes that can influence healing or inadvertently. Therefore, there are no professional advice about how managing diabetes affects the healing of chronic wound.[27] An extensive range of physiological and molecular mechanisms, such as inflammation, cell proliferation, angiogenesis, collagen deposition, and re-epithelialization, are involved in wound healing. The invasion of inflammatory cells at the wound site is one of the early phases of the wound healing process. This inflammatory response involves the increase of macrophages, which are significant contributors to healing since monocyte macrophage depletion results in delayed re-epithelialization, decreased collagen deposition, poor angiogenesis, and decreased cell proliferation. Prolonged inflammatory response in wounds is linked to poor healing.[27,28]

The results of the study demonstrated the ability of *Glacillaria sp.* gel from kajhu Aceh Besar to accelerate clinical wound healing in rats induced diabetes with streptozotocin, histological findings showed a higher number of fibroblasts, greater epithelialization, and more regular collagen fibers, approaching the positive control group which hyaluronic acid gel was applied.
Alkaloids, steroids, sapnins, phenolics, terpenoids, and tannins are all known to be present in the red algae from Kajhu aceh Besar. (Table 1) The presence of flavonoids is indicated by a colour shift from red to yellow to orange. The presence of alkaloids is indicated by the production of an orange precipitate in Tube 1 and a yellowish precipitate in Tube 2. The presence of saponins in the studied extract was shown by the formation of stable foam. The production of greenish brown or dark blue stains indicates the presence of visible tannins. The presence of phenolic chemicals in the seaweed extract is indicated by the production of a green or blue-green colour. Steroids are indicated by a blue to green colour, whilst triterpenoids are indicated by a brownish or purple ring at the boundary of the two solvents.
Protein synthesis, wound contraction, cellular infiltration, neo-vascularization, and epithelialization are all enhanced by alkaloids.[29] The TGF-family, retinoids, and glucocorticosteroids all affect cell proliferation and differentiation via the same route.[30] Flavonoids have been demonstrated to stimulate collagen cross-linking, minimise soluble collagen degradation, speed up the conversion of soluble collagen to insoluble collagen, and inhibit soluble collagen catabolism. Collagen is the most abundant protein in the extracellular matrix and is a component that leads to enhanced wound strength and epithelialization.[31]

Table 1; Phytochemical Test

Metabolite	Red Algae
Alkaloid	+
Steroid	+
Terpenoid	+
Saponin	+
Flavonoid	+
Fenolik	+
Tannin	+

Table 2: GCMS test *Gracilaria sp*. from Kajhu Aceh Besar

QTY	Phytochemicals	%
99	Hexadecanoic acid	39.80
99	(9e)-9-octadecanoic acid	11.34
68	1,2-Benzenedicarboxylic acid	6.70
64	Erythritol	6.49
98	Cholesterol	5.12
91	(9e)-9-octadecanoic acid	4.67
53	1-nonadecene	3.83
64	Ethylamine, N,N-dimethyl-2-[4-(chloromethyl)phenoxyl]	3.21
62	15-Hydroxypentadecanoic acid	2.54
92	Dibutyl phthalate	2.40
38	N,N-Dimethyl-10-undecen-1-1amine	2.13
56	2-Heptadecenal	1.95
92	Heptadecane	1.29
42	Piperazine, 1-methyl-4[2-(ptolylsulfonyl)ethyl]	1.11
25	1,6-Cyclododecadiyne	1.00

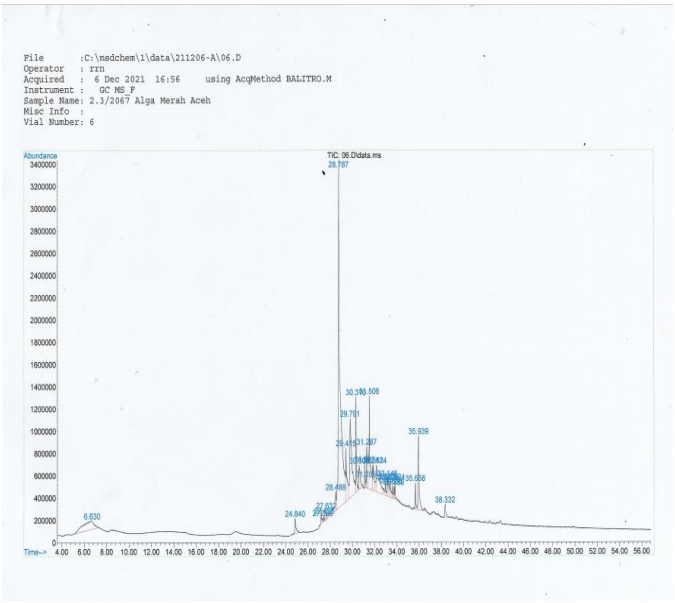

Figure 3: Total Ion Chromatogram (TIC) of Red Algae

Tannins have an inhibitory effect on Staphylococcus aureus and encourage the growth and migration of fibroblasts into the wound. Tannins can hasten re-epithelialization, speed up wound healing, enhance angiogenesis, and promote wound infection recovery. A class of water-soluble metabolites called tannic acid can function as a combination of macromolecules and metal ions that possesses anti-oxidant, anti-microbial, and therapeutic properties. Tannins are antioxidants with astringent and antibacterial effects. Tannins assemble into complexes with proteins and/or polysaccharides to provide an extra layer of protection for damaged epithelium cells. Tannins can promote intercalary growth on the membrane, fibroblast cell adhesion, and tissue regeneration. The capacity of tannins to function as an astringent, which encourages the elimination of water from cells and causes fibres to constrict, is thought to be the cause of their therapeutic effects.[32 ,17]
Phenolic chemicals also have favourable effects on wound healing through a variety of pathways such as antioxidant, antibacterial, anti-inflammatory, collagen synthesis stimulation, cell proliferation, and angiogenic actions. The improved wound healing activity of phenolics is mostly owing to their antioxidant activities and free radical oxidative scavenging. By preventing reactive oxygen species, phenols can aid in wound healing by acting as an anti-bacterial, anti-inflammatory, and antioxidant.[33, 16, 34] In wound surgery, triterpenes induce a reduction in closure time, and this effect has been reported in nearly all wound types. Triterpenes also modulate ROS production in the wound microenvironment, accelerating the tissue repair process. Triterpenes can also induce cell migration, cell proliferation and collagen deposition.[35]
Glacilaria sp. GCMS analysis revealed that the content of *Glacilaria sp*. from Kajhu Aceh Besar contains (Tabel 2 and Figure 3) 39.80 percent Hexadecanoic acid. (9e)-9-octadecanoic acid, was found in the second order at a concentration of 11.34 percent. 1,2-Benzenedicarboxylic acid, came in third with 6.70 percent, followed by Erythritol at 6.49 percent and Cholesterol at 5,12 percent. Furthermore, (9E)- (9e)-9-octadecanoic acid, 4.67 percent. Our research found 3.83 percent 1-nonadecene. Ethylamine, N, N-dimethyl-2-[4-(chloromethyl)phenoxyl] 3.21 percent, 15-Hydroxypentadecanoic acid 2.54 percent, 2.40 percent Dibutyl phthalate, 2.13 percent N, N-Dimethyl-10-undecen-1-1amine, 1.95 percent 2-Heptadecenal, 1.29 percent Heptadecane, 1.11 percent Piperazine, 1-methyl-4[2-(ptolylsulfonyl)ethyl] and 1 percent 1,6-cyclododecadiyne.
The largest content of red algae from Kajhu Aceh is 39.80 percent Hexadecanoic acid, Hexadecanoic acid has other names Cetylic acid and palmitic acid, with the chemical formula $C_{16}H_{32}O_2$. Palmitic acid has a 16-carbon backbone and is a saturated long-chain fatty acid. Natural sources of palmitic acid include palm oil and palm kernel oil, as well as butter, cheese, milk, and meat. Proliferating cells must duplicate all of their cellular components, which involves nucleotide, amino acid, and lipid production. Palmitic acid synthesis requires 7 ATP molecules, 16 carbons from 8 acetyl-CoA molecules and 14 NADPH molecules, totaling 28 electrons. A 16-carbon fatty acyl chain requires one glucose molecule, which when fully oxidised can yield five times the required ATP, but NADPH requires seven glucose molecules. This significant asymmetry is only partially balanced by the expenditure of three glucose molecules in the production of acetyl-CoA to meet the acyl chain's carbon requirements. The amount of glucose required for cell growth is unrelated to carbon catabolism for ATP synthesis; in fact, this would be advantageous. The amount of glucose necessary for cell growth has nothing to do with carbon catabolism for ATP synthesis. In fact, increasing the ATP/ADP ratio would substantially hamper the flux through the glycolytic intermediate, lowering acetyl-CoA and NADPH synthesis essential for macromolecule formation. To maximize ATP production, this mitochondria completes the conversion of glucose to CO_2 via oxidative phosphorylation. Lipid metabolism gives membranes the ability to divide cells, reproduce biologically, and exchange intracellular membrane substances. Additionally serving as messengers in the signalling and molecular recognition processes are fatty acids[36] This makes the cell environment of the oral mucosa that is in the wound healing stage a lot of energy for fibroblast proliferation in wound healing after application of *Glacilaria sp.* gel containing palmitic acid. The palmitic acid content of the *Glacilaria sp.* that we tested, showed a

higher number of fibroblasts than those given hyaluronic gel and placebo. (Figure 5)

$C_{18}H_{34}O_2$ or $C_8H_{17}CH=CH(CH_2)_7COOH$, 9-Octadecenoic acid, synonym oleic acid Carbopol 934P, As a thickening agent in gels, suspensions, and emulsions, a high molecular weight polymer of acrylic is used. A significant amount of research has recently been published on the mucoadhesive properties of carbopol, establishing it as a useful adjuvant for bio adhesive drug delivery systems due to its ability to prolong organ residence time and increase contact time with absorbing mucosa, resulting in improved drug absorption. It is a biodegradable, mucoadhesive, and environmentally friendly polymer. Carbopol's oleic and linoleic acids hasten wound healing and skin tissue regeneration by causing considerable wound contraction, faster re-epithelialization, and intense disposition of collagen bundles with a high content of active fibroblasts. [37] In this study red algae containing oleic acid 11.34 percent showed clinically faster healing of oral mucosal wounds of *Rattus norvegicus* induced by streptozotocin with a wound that has completely closed on day 7 while the negative control group on the 7th day the wound has not closed, and in the positive control group on the 7th day the wound has closed with a small ulcer (Figure 4), histologically the number of fibroblasts was more numerous with thicker collagen with a more organized pattern (Figure 5) and epithelialization was thicker, compared to the positive control group which contained hyaluronic acid and the control group which contained distilled water. (Table 3)

Erythritol was also found in the *Glacilaria sp.* gel that we used to treat the *Rattus norvegicus* oral mucosal wounds that we studied. Erythritol functions as an endothelial protector and antioxidant.[38] Antioxidants can also affect collagen synthesis by increasing collagen proliferation [39] through its ability to make *Glacilaria sp.* gel containing erythritol that we used in this study was able to accelerate wound healing by accelerating the proliferative phase and early remodeling phase in *Rattus norvegicus* oral mucosal incision wound induced by streptozotocin.

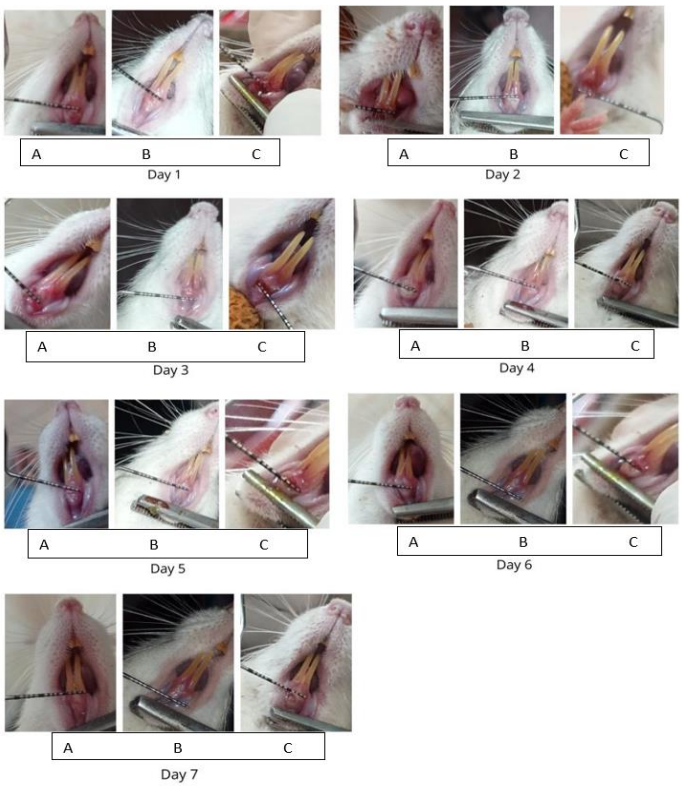

Figure 4: Oral mucosa wound healing in streptozotocin induced diabetes in rats from day 1 until day 7, group A *Glacilaria* application group B Gengigel application Group C placebo application

Positive effects on cholesterol metabolism and triglyceride derivatives have immune cell-modulating capabilities, with immune-modulating action involved in the polarization of bone marrow-derived macrophages to an anti-inflammatory phenotype. Cholesterol promotes fibroblast growth, which aids in wound healing and regeneration. By decreasing superoxide dismutase (SOD) and nitric oxide (NO), the transforming growth factor-beta is elevated [40] The *Glacilaria* we use also contains cholesterol, This demonstrates that its content influences the number of oral mucosal incision wound fibroblasts during the proliferative phase. (Figure 5)

Pistacia atlantica Gel, which includes oleic acid fatty acids, was utilised in another study by Seyed Ahmadreza Hamid et al. and had a proinflammatory effect on the healing of cutaneous wounds. Oleic and linoleic acid-based fatty acid combinations have been used to treat and prevent pressure ulcers.[41] Topical fatty acid use also promotes hydration, suppleness, and skin breakdown prevention. The results demonstrate that total lymphocyte count was reduced in both the 5% and 10% Bene gel groups twenty-one days after injury when compared to the control and base gel groups, but only 10% in the 10% Bene treated group when compared to the 5% Bene, control, and base gel groups. Topical administration of Bene oil gels (particularly Bene 10%) resulted in enhanced re-epithelialization with continuous stratum basalis and mature granulation tissue and adnexa (hair follicles and sweat gland) as compared to the control and base gel groups. Lesions in the Bene oil gel-treated groups had better cosmetic results during wound repair than the control and base gel groups. Treatment with 10% Bene oil gel significantly enhanced tensile strength, ultimate stress, yield strength, and stiffness at 21 days post-injury as compared to the control and base gel groups. The collagen fibres showed a more organized pattern and tissue alignment was better than in the control and base gel-treated groups at the same stage. Gel groupings are advantageous. The total fibroblast count was noticeably higher.[42] Research from Hakim et al. found that red algae from Pulo Aceh had a positive effect on wound healing clinically and histologically, with an increase in the amount of collagen in normal rats' oral mucosal lesions after topical application gel made from red algae extract.[14]

Fibroblasts move to the wound area via chemo-attractants such as PDGF, TGF- ß, FGF, interleukin-1 beta (IL-1), and tumor necrosis factor-alpha, which platelets and macrophages create in the temporary matrix as part of the inflammatory response. Fibroblasts move to the wound site, multiply, and create MMPs, which damage ECM (extracellular matrix) temporarily. Through numerous integrins positioned in the focal adhesion (Fas), fibroblast activity lowers and binds the fibrin clot to the lesion. Fibroblasts generate granulated tissue that heals the wound by replacing the temporary ECM with a new ECM composed of collagen, proteoglycans, hyaluronic acid, glycosaminoglycans, and fibronectin. Type 3 collagen is rapidly produced, with an initial matrix that acts as a barrier to pathogens and serum and fluid loss, before being degraded by proteases and redesigned by fibroblasts to be replaced by type 1 collagen, which has a much higher tensile strength but takes much longer to deposit. [43]

The *Glacilaria sp.* group from Aceh was significantly different from the negative control group but not significantly different from the positive control group. This can be caused because the largest content of *Glacilaria sp.* from Kajhu Aceh is 39.80 percent Hexadecanoic acid. Hexadecanoic acid has other names Cetylic acid and palmitic acid, with the chemical formula $C_{16}H_{32}O_2$. Palmitic acid is a long-chain saturated fatty acid with a 16-carbon backbone. Palmitic acid aids in wound healing during the proliferative and remodeling phases. [42] According to our findings, wounds treated with red algae gel had a greater number of fibroblasts that epithelialized more on hematoxylin-eosin examination, more collagen that was more organized on mason trichome examination, and collagen type 3 was detected clearer and with a greater amount of staining on immunohistochemical examination. In this study, type 3 collagen is produced more quickly and with better structure. This type 3 collagen's low initial matrix acts as a barrier to pathogens and prevents fluid loss. Type 3 collagen is then destroyed by proteases and redesigned by fibroblasts to be replaced by type 1 collagen. The subsequent formation of type 1 collagen will also be quicker, improving the wound's tensile strength.[19]

Conclusion

The application of red alga gel from Kajhu to diabetic-induced rat oral mucosal lesions altered the proliferative phase by increasing the number of fibroblasts, the thickness of epithelialization, and collagen synthesis at the early remodeling stage

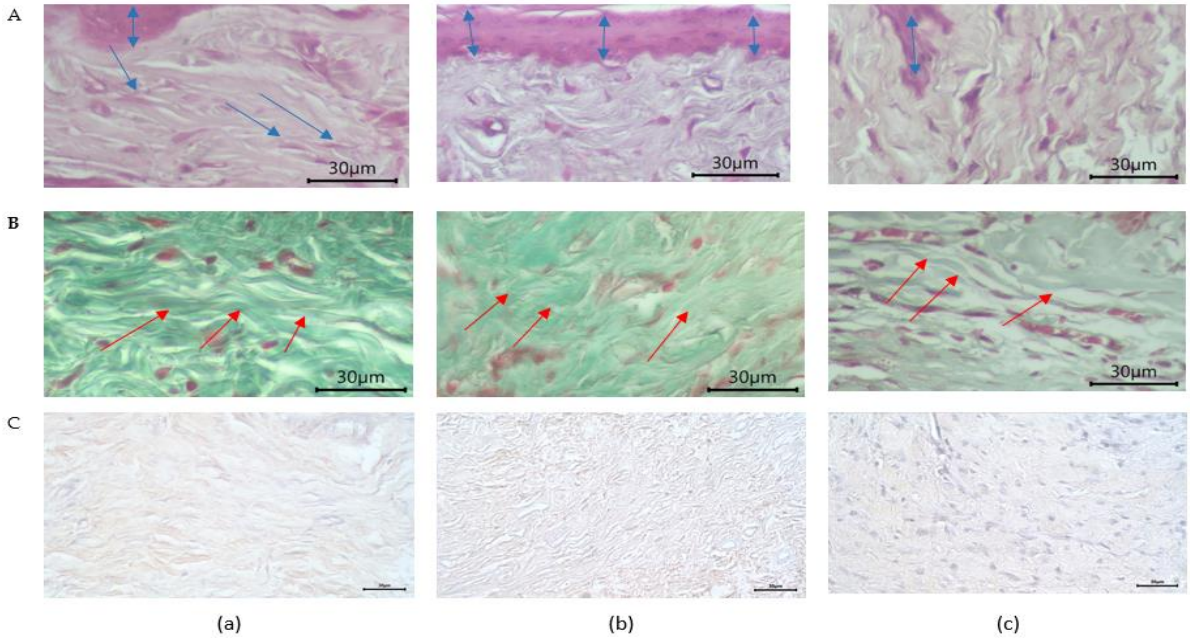

Figure 5: Histologically feature Comparison of (A) epithelialization (blue double arrow), fibroblast cell count (blue arrow), (B)collagen Mason Trichrome (red Arrow)(C) Immunohistochemistry collagen (Brown)type 3 from (a) Red Algae, (b) Positive control and (c) Negative Control

Table 3: The results of sample observations in the positive control group (K+), the extract group (P), negative control group (K-)

The Number of Fibroblast	**Mean**	**Std Deviation**	***P-value*** **Normality[a]**	***P-value*** **Homogeneity[b]**	**ANOVA**	**Tukey HSD**
				0.089	0.049[C]	
(K+) Control +	13.340	0.495	0.828			0.386
(P)	16.640	2.391	0.147			
(K-) Control -	11.000	0.414	0.937			0.030
The Collagen Type 3	Mean	Std Deviation	*P-value* Normality[a]	*P-value* Homogeneity[b]	ANOVA	Tukey HSD
				0.158	0,000[c]	
(K+) Control +	742182.40	1134.288	0.465			0.000
(P)	512546.60	1116.033	0.551			
(K-) Control -	206147.4	616.403	0.102			0.000
The epithelialization	Mean	Std Deviation	*P-value* Normality[a]	*P-value* Homogeneity[b]	ANOVA	Tukey HSD
				0.254	0.058[C]	
(K+) Control +	30.436	4.424	0.530			0.391
(P)	24.680	1.580	0.236			
(K-) Control -	19.036	2.179	0.440			0.404

a Data distributed normally if the p-value > 0.05
b Homogeny if the p-value > 0.05
c Significant if the p-value < 0.05

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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