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Original Article

Characterization of red algae (*Gracilaria verrucosa*) on potential application for topical treatment of oral mucosa wounds in *Rattus norvegicus*

Rachmi F. Hakim[1,2], Rinaldi Idroes[1,3,4*], Olivia A. Hanafiah[5], Binawati Ginting[4], Pati Kemala[1], Fakhrurrazi Fakhrurrazi[2], Noviandi I. Putra[2], Ghina A. Shafira[2], Yenni Romadhoni[2], Khaerunisa Destiana[2] and Muslem Muslem[6]

[1]Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia; [2]Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh, Indonesia; [3]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia; [4]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh, Indonesia; [5]Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia; [6]Department of Chemistry, Faculty of Science and Technology, Ar-Raniry State Islamic University, Banda Aceh, Indonesia

*Corresponding author: rinaldi.idroes@usk.ac.id

Abstract

Wound healing in the mouth has its challenges due to masticatory movements and the presence of bacteria that can inhibit its process. The aim of this study was to analyze the contents of red algae (*Gracilaria verrucosa*) from Indonesia, and its potential as a wound-healing agent for oral wounds using animal model. Red algae content was determined by phytochemical tests and gas chromatography-mass spectroscopy (GC-MS). The wound was made by making an incision on the gingival mucosa of *Rattus norvegicus* and the parameters assessed were bleeding time, number of fibroblast cells, and time of wound closure. Three doses of *G. verrucosa* gel were used (2.5%, 5%, and 10%) and the gels were applied twice a day, at 6:00 and 18:00. Application was carried out topically by applying 0.1 ml of gel to the incision wound using a 1 mL syringe. Our phytochemical test indicated that the *G. verrucosa* contained alkaloids, steroids, flavonoids, and phenols. The dominant contains of the *G. verrucosa* were glycerol (36.81%), hexadecenoic acid (20.74%), and cholesterol (7.4%). The hemostasis test showed that the 2.5% *G. verrucosa* extract gel had the shortest bleeding time, 33.98±2.66 seconds. On the seventh day of the initial proliferation phase, the number of fibroblasts was not significantly different among groups. On day 14, the number of fibroblasts was only significantly different between 10% *G. verrucosa* and untreated group (p=0.007). On day 28, however, both 5% and 10% *G. verrucosa* were significantly higher compared to untreated group, both had p=0.010. Daily clinical examination showed that animals that were given 2.5% and 5% of *G. verrucosa* extract gel experienced wound closure on day 10. Animals treated with 10% of extract gel, all wounds healed on day 9. This study suggested that *G. verrucosa* extract could accelerate wound closure and wound healing.

Keywords: GC-MS, hemostasis, In vivo, proliferation, wound-healing

Introduction

An injured body will go through wound-healing stages, including the hemostasis, inflammation, proliferation, and remodeling stages [1]. Hemostasis is the initial phase of the wound-healing process [2,3] and this stage regulates blood loss after tissue damage [3,4]. Some body responses

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such as blood coagulation and fibrinolysis occur during this hemostasis stage [5]. The inflammatory stage involves the activation of multiple signaling pathways that result in the release of cytokines and chemokines by cells present in the wound [6]. These molecules attract immune cells such as neutrophils, monocytes, macrophages, mast cells, and T cells to the wound site, fighting pathogens and accelerating the healing process [7]. In the proliferation phase, endothelial cells, fibroblasts, and epithelial cells migrate into the wound site to regenerate tissue. During this phase, highly vascular and loose granulation tissue is formed, provided by fibroblasts and endothelial cells to provide structural and nutritional support to the wound site [8].

Many studies have examined wound healing on the skin [9-11], but there are still few studies on wounds in the oral mucosa. Wound-healing challenges in the oral cavity include the presence of microorganisms that can inhibit the inflammatory phase and blood clots that are more difficult due to chewing activities. Natural materials have become popular and recommended by researchers for medicinal purposes [12-14]. Natural materials have good bioactivity as antimicrobial [15,16], antiviral [17,18], antibacterial [19-21], antibiofilm [22], antihypercholesterolemia [23], and as antioxidants [24,25]. Natural materials have advantages over synthetic materials as they are safe and have much lower side effects [26,27]. In this study, the natural product proposed as a wound healer for oral mucosa is *Gracilaria verrucosa*.

G. verrucosa is a high-value commercial red algae species (Rhodophyta) with a reputation as a food source for human and fish. This species ranks highest among approximately 452 algae growing in Indonesian waters [28,29]. Previous studies have reported that red algae contain amino acids [30-32], fatty acids [29,33-35], saponins, triterpenoids, steroids, flavonoids, tannins, phenols [4], alkaloids, potassium, sodium, calcium, phosphorus [29], zinc, copper, magnesium, iron [36,37], vitamins A, B, C [38], quinones, monoterpenoids, and sesquiterpenes [39, 40]. Based on the variation of natural contents in red algae, this species is believed to have great potential as an agent for oral mucosa healing.

The aim of this study was to investigate the compounds in red algae that grow in West Java, Indonesia, and their potential as wound-healing agent of oral area. The wound healing activity of the ethanol extract of red algae was tested on *Rattus norvegicus*. The role of the ethanol extract of red algae was tested at three stages of wound healing processes: hemostasis (bleeding time calculation), proliferation (fibroblast cell count), and clinical wound healing stage (wound closure duration).

Methods

Red algae samples

G. verrucosa specimens were collected from Tambaksari, Tirtajaya, Karawang, West Java, Indonesia (6° 00'33.1 "S 107° 14'07.2" E). *G. verrucosa* has the appearance of being brown, dark olive, or dark crimson. Thalli were 25–30 cm tall, upright, and cylindrical. The main axis was 1.5–2 mm in diameter, with 3–4 orders of lateral branches of varying sizes spaced at irregular intervals in an alternating or unilateral pattern. The branches were typically shortened at the base. The outer cortical cells were radially elongated and ovoid and range in size from 4.5–7 mm. The taxonomic determination was carried out by experts from the Indonesian Institute of Science (BRIN) with registered number B-896/V/DI.05.07/3/2022.

Sample extraction

The red algae were macerated for five days in containers using ethanol at a concentration of 96% that was shielded from bright sun (this was done to minimize reactions or discoloration that was catalyzed by light). After that, the samples were concentrated using a rotary evaporator (Butchi Rotavor®, Switzerland) so that a pure ethanol extract of *G. verrucosa* could be obtained.

Phytochemical test

The phytochemicals contained in *G. verrucosa* ethanol extract were tested. Flavonoids (Shinoda test), steroids and terpenoids (Liebermann-Buchard test), alkaloids (Dragendorff test), saponins (foam test), tannin (ferric chloride test), and phenolic tests were performed [41-43].

Gas chromatography-mass spectroscopy (GC-MS) analysis

Gas chromatography-mass spectroscopy (GC-MS) (Agilent Technologies 7890, Santa Clara, CA, United States) was used to determine the compounds of ethanol extract of *G. verrucose* [44]. Gas chromatography was used and it has a 30 m SPB-50 column with a 0.25 mm inside diameter and a 0.25 m film thickness. The interface temperature was set to 250°C, and the injection temperature was 230°C. The temperature of the ion source was set to 200°C. Helium was used as the carrier gas, and the flow rate was kept steady at 1 ml/min. The temperature program used was isothermal heating for five minutes at 70°C, then increasing the oven temperature by 5°C/minute to 310°C, and finally heating for one minute at 310°C. The mass spectrum was recorded at two scans per second and a range of 50–600 m/z. A mass laboratory program was used to look at the chromatogram and mass spectrum. In a mass laboratory method, retention time and mass spectra were used to count the number of peaks of metabolites automatically. Microsoft Excel was used to enter the algorithm. The putative compounds were identified using the National Center for Biotechnology Information (NCBI) database [45].

Preparation of red algae gel

The ethanol extract of *G. verrucosa* was combined with 25 g of natrium carboxymethyl cellulose (Na-CMC), 25 g of propylene glycol, 50 g of glycerin, and 6 mg of nipagin. The solution was stirred until homogenous. The extract was then dissolved in deionized water at 50°C. In a different mortar, 1.25 g of Na-CMC mucilaginous material was added and stirred for 15 minutes until a gel mass was produced. The red algae extract gel was made in four different concentrations: 2.5%, 5%, and 10%. All prepared gels were stored in vials.

Rattus norvegicus gingiva incision

The *R. norvegicus* were studied for two stages of wound healing: the hemostasis stage (calculation of bleeding time) and the proliferation stage (total fibroblast number). The *R. norvegicus* weighed on average between 200 and 250 g, ten weeks old, and were in excellent condition with good movement characteristics and dense fur. Animal experiments were conducted at the Animal Experiment Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. The labial gingiva underneath the two mandibular anterior teeth was chosen as the targeted area. Each animal was anesthetized with a single intramuscular injection of 100 mg/kg xylazine hydrochloride and 5 mg/kg ketamine hydrochloride as the initial step of anesthesia. The incision was produced by 5 mm in length and bone depth with a scalpel and blade No. 11.

Bleeding time calculation

Five animals, each group with four groups (3 doses of *G. verrucosa* gel, 2.5%; 5%; and 10% and negative control (0%)), were utilized for the bleeding time test. The negative control group's bleeding duration was calculated immediately by placing blotting paper near the wound without affecting hemostasis. Using a QQ chronometer (Citizen Watch, Japan), the bleeding time was determined until the blotting paper could no longer absorb the blood. After the incision, the *G. verrucosa* gel was applied to the wound in the treatment groups, and the bleeding time was immediately measured. The duration of bleeding was determined by the same method as the negative control group.

Histological examination

Experimental animals' oral mucosa wound sites were sliced along 5 mm and preserved in a 10% formaldehyde phosphate solution for 18–24 hours. The samples were processed to make histological samples with a thickness of 5 mm. The samples then stained with hematoxylin and eosin.

Determine of fibroblasts

In order to examine the number of fibroblasts, 30 *R. norvegicus* were divided into two groups: 5% *G. verrucosa* gel group and the negative control group. Fibroblasts from the gingiva wound were observed and counted in 7^{th}, 14^{th} and 28^{th} day using a microscope (Meiji Techno Microscope,

Japan), digital camera DP-12 (Olympus Camera, US), and Top View software with 400x magnification and five fields of view.

Wound closure time

The reduction in wound diameter of the incision wound was monitored daily in each group using a periodontal probe (UNC-15) (Kohler Medizintechnik, Germany). When the wound diameter was 0 mm or when full wound closure occurred, the wound was classified as healed. After completion of treatment, anesthesia was injected into all animals before cervical dislocation. After a histological examination of the samples, *R. norvegicus* was buried.

Data analysis

Fibroblast cell count data were analyzed using the SPSS software, version 25.0 (IBM Corp., Armonk, NY, USA). The data normality test was performed using the Shapiro-Wilk test and the variance homogeneity test using Levene's test. The analysis of variance (ANOVA) was used and followed by a post-hoc test with the least significant difference (LSD) test to compare the mean of the bleeding time and fibroblast numbers among and between groups.

Results

Phytochemical analysis

The results of the phytochemical tests of *G. verrucosa* are represented in **Table 1**. The phytochemical tests showed that the ethanol extract of *G. verrucosa* contained alkaloids, steroids, flavonoids, and phenols.

Table 1. Phytochemical tests of *Gracilaria verrucosa*

Metabolite	Reagent test	Result
Alkaloids	Mayer, Wagner, Dragendorf	Positive
Steroids	Liebermann-Burchard	Positive
Terpenoids	Liebermann-Burchard	Negative
Saponin	Shakes	Negative
Flavonoids	HCl solution and Mg	Positive
Phenolic	FeCl3	Positive

GC-MS analysis

The results of GC-MS are represented in **Figure 1** and **Table 2**. The data presented in **Table 2** are all putative compounds that have a similarity more than 90% with NCBI database, except for glycerol because it has the highest percentage. The highest content percentages of *G. verrucosa* ethanol extract was glycerol (36.81%) and hexadecanoic acid (20.7%) followed by minor ones of cholesterol, (9e)-9-octadecanoic acid, E,e-10,12-hexadecadien-1-ol acetate, phytol, and 2-amino ethanethiol hydrogen sulfate (ester) (with the content amounts of 7.40; 4.35; 3.27; 1.51; 1.38; and 1.00%, respectively).

Table 2. Putative chemical contents of *Gracilaria verrucosa* identified by GCMS

Quantity	Phytochemicals	Retention time	%	Molecule formula	Class of compound
83	Glycerol	10.3	36.81	C3H8O3	Organooxygen compounds
99	Hexadecanoic acid	28.8	20.74	C16H32O2	Fatty acyls
99	Cholesterol	36.0	7.40	C27H46O	Steroids
99	(9e)-9-Octadecenoic acid	29.7	4.35	C20H38O2	Fatty acyls
96	E,e-10,12-hexadecadien-1-ol acetate	30.6	3.27	C18H32O2	Fatty acyls
92	(9E)-9-octadecenoic acid	32.1	1.38	C18H34O2	Fatty acyls
96	2-aminoethanethiol hydrogen sulfate (ester)	30.0	1.00	C13H29NO3S2	Organic thiosulfuric acid

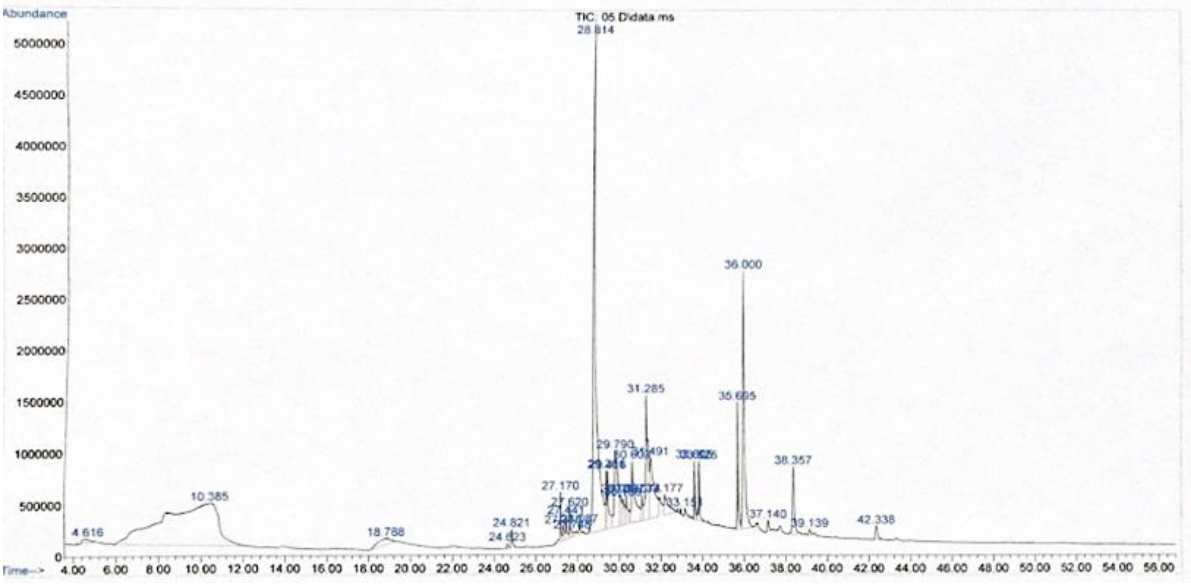

Figure 1. Total ion chromatogram (TIC) of *Gracilaria verrucosa*.

Hemostasis stage

Calculations of the bleeding time in experimental animals using *G. verrucosa* extract are presented in **Figure 2**. The experimental group of animals that were not given sample treatment had the longest bleeding time compared to other experimental animal groups. The *G. verrucosa* extract sample with a concentration of 2.5% had the shortest average bleeding time, 33.98±2.66 seconds. The bleeding time of the experimental animals given *G. verrucosa* extract at concentrations of 5% and 10% was relatively the same, 36.4±2.71 and 35.55±5.42 seconds, respectively (**Figure 2**). The ANOVA results indicated the mean of the bleeding time was different significantly among groups ($p<0.0001$). Post-hoc with LSD indicated that the best dose for bleeding time was 2.5% group, with all three concentrations had significant shorter bleeding time compared to the control (**Table 3**).

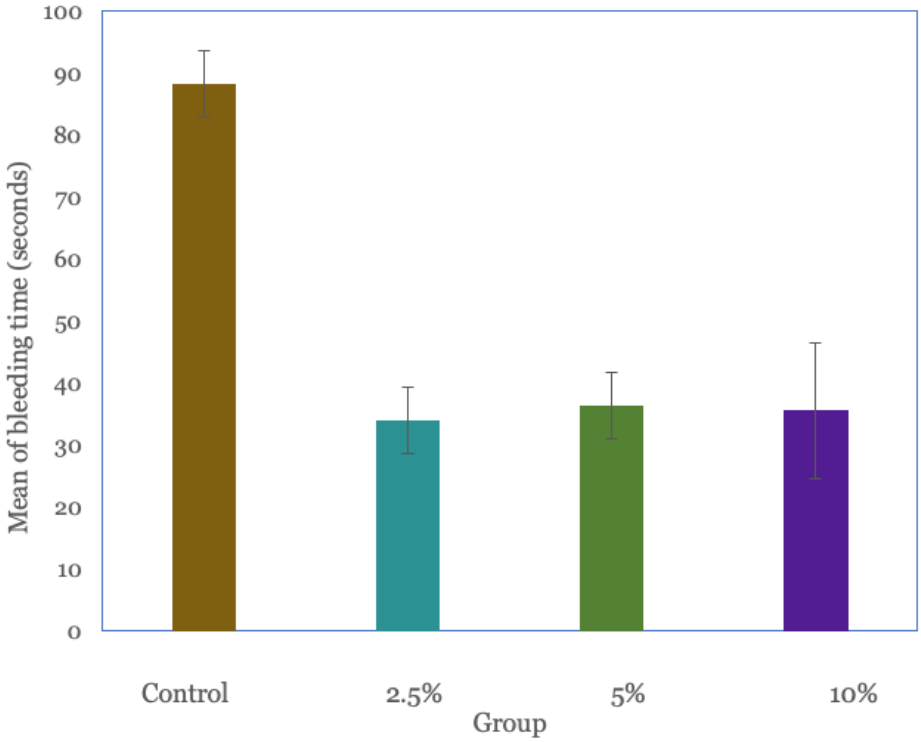

Figure 2. The effect of the concentration of *Gracilaria verrucosa* extract gel on bleeding time.

Table 3. Post-hoc analysis using least significance different (LSD) test for bleeding time

Day and group	Groups			
	Negative control	2.5% *G. verrucosa*	5% *G. verrucosa*	10% *G. verrucosa*
Control	-	0.009*	0.014*	0.003*
2.5% *G. verrucosa*	0.009*	-	0.873	0.748
5% *G. verrucosa*	0.014*	0.873	-	0.630
10% *G. verrucosa*	0.003*	0.748	0.630	-

* Statistically significant at $p<0.05$

Proliferation stage

The proliferative stage of wound healing can be observed from the number of fibroblast cells. The histological examination of fibroblast cells has been presented in **Figure 3**. The number of fibroblast cells in each group and comparisons of fibroblast cells among and between groups in three different time points are presented in **Table 4** and **Table 5**.

Table 4. Comparison of fibroblast number among groups in three different time points

Day and group	Mean of fibroblast number	Std deviation	*p*-value [a]
7th day			0.754
Control	9.80	2.17	
2.5% *G. verrucosa*	9.60	4.45	
5% *G. verrucosa*	10.60	5.03	
10% *G. verrucosa*	7.80	4.44	
14th day			0.039*
Control	11.96	0.89	
2.5% *G. verrucosa*	12.96	2.25	
5% *G. verrucosa*	13.92	1.50	
10% *G. verrucosa*	14.96	1.11	
28th day			0.027*
Control	1.36	0.41	
2.5% *G. verrucosa*	6.56	4.12	
5% *G. verrucosa*	7.32	2.13	
10% *G. verrucosa*	7.36	4.48	

[a] Analyzed with ANOVA
* Statistically significant at $p<0.05$

Table 5. Post-hoc least significance different (LSD) test of comparisons of fibroblast number among groups in three different time points

Day and group	Groups			
	Negative control	2.5% *G. verrucosa*	5% *G. verrucosa*	10% *G. verrucosa*
14th day				
Control	-	0.316	0.059	0.007*
2.5% *G. verrucosa*	0.316	-	0.335	0.055
5% *G. verrucosa*	0.059	0.335	-	0.298
10% *G. verrucosa*	0.007*	0.055	0.298	-
28th day				
Control	-	0.022	0.010*	0.010*
2.5% *G. verrucosa*	0.022	-	0.715	0.700
5% *G. verrucosa*	0.010*	0.715	-	0.985
10% *G. verrucosa*	0.010*	0.700	0.985	-

* Statistically significant at $p<0.05$

Wound healing

Measurements of daily clinical examinations on treated *R. norvegicus* wounds have been presented in **Table 6**. Based on the data obtained, it is known that the higher the concentration of red algae extracts in the gel, the faster the wound closure. The group of experimental animals treated with gel with 10% sample extract was the group whose wounds closed faster (**Figure 4**).

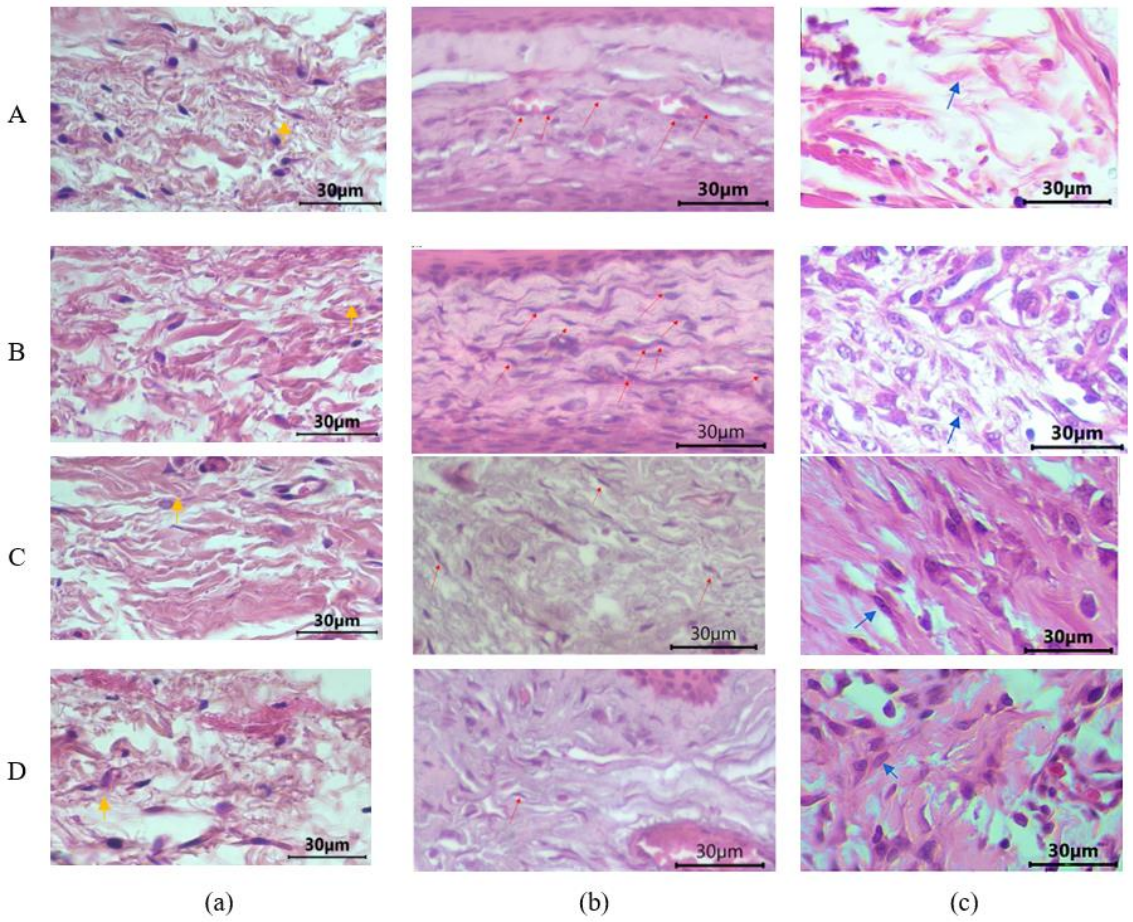

Figure 3. Comparison of fibroblast cell count between 2.5% *Gracilaria verrucosa* (A), 5% *G. verrucosa* (B), *G. verrucosa* group 10% (C), and control group (D) in three different time points: 7th day (a), 14th day (b) and 28th day (c). Yellow, red and blue arrows indicate fibroblast cells on days 7, 14 and 28, respectively.

Table 6. Measurement daily clinical examination of *Rattus norvegicus* wound applying red algae extract gel

Gracilaria verrucosa extract gel	*Rattus norvegicus*	Wound length reduction (mm)											
		1	2	3	4	5	6	7	8	9	10	11	12
2.5%	1	5	5	4	4	3	3	1	1	1	0	0	0
	2	5	5	4	4	3	3	2	2	1	1	0	0
	3	5	5	4	3	3	3	2	1	1	0	0	0
	4	5	5	4	4	3	3	2	1	1	0	0	0
	5	5	5	4	4	3	2	1	2	1	1	0	0
5%	6	5	5	4	4	2	2	1	1	1	0	0	0
	7	5	5	4	3	3	2	2	1	1	0	0	0
	8	5	5	4	4	2	2	1	1	1	0	0	0
	9	5	5	4	3	2	2	1	1	1	0	0	0
	10	5	5	4	4	3	2	1	1	1	0	0	0
10%	11	5	5	3	3	2	2	1	1	1	0	0	0
	12	5	5	3	3	2	2	1	1	1	0	0	0
	13	5	5	3	3	2	2	1	1	1	0	0	0
	14	5	5	3	3	2	2	1	1	0	0	0	0
	15	5	5	3	3	2	2	1	1	1	0	0	0
Control	16	5	5	4	4	3	3	2	2	2	1	1	0
	17	5	5	4	4	4	3	2	2	2	1	0	0
	18	5	5	4	4	4	3	2	2	2	1	0	0
	19	5	5	4	4	4	3	2	2	2	1	1	0
	20	5	5	4	4	4	3	3	2	2	1	0	0

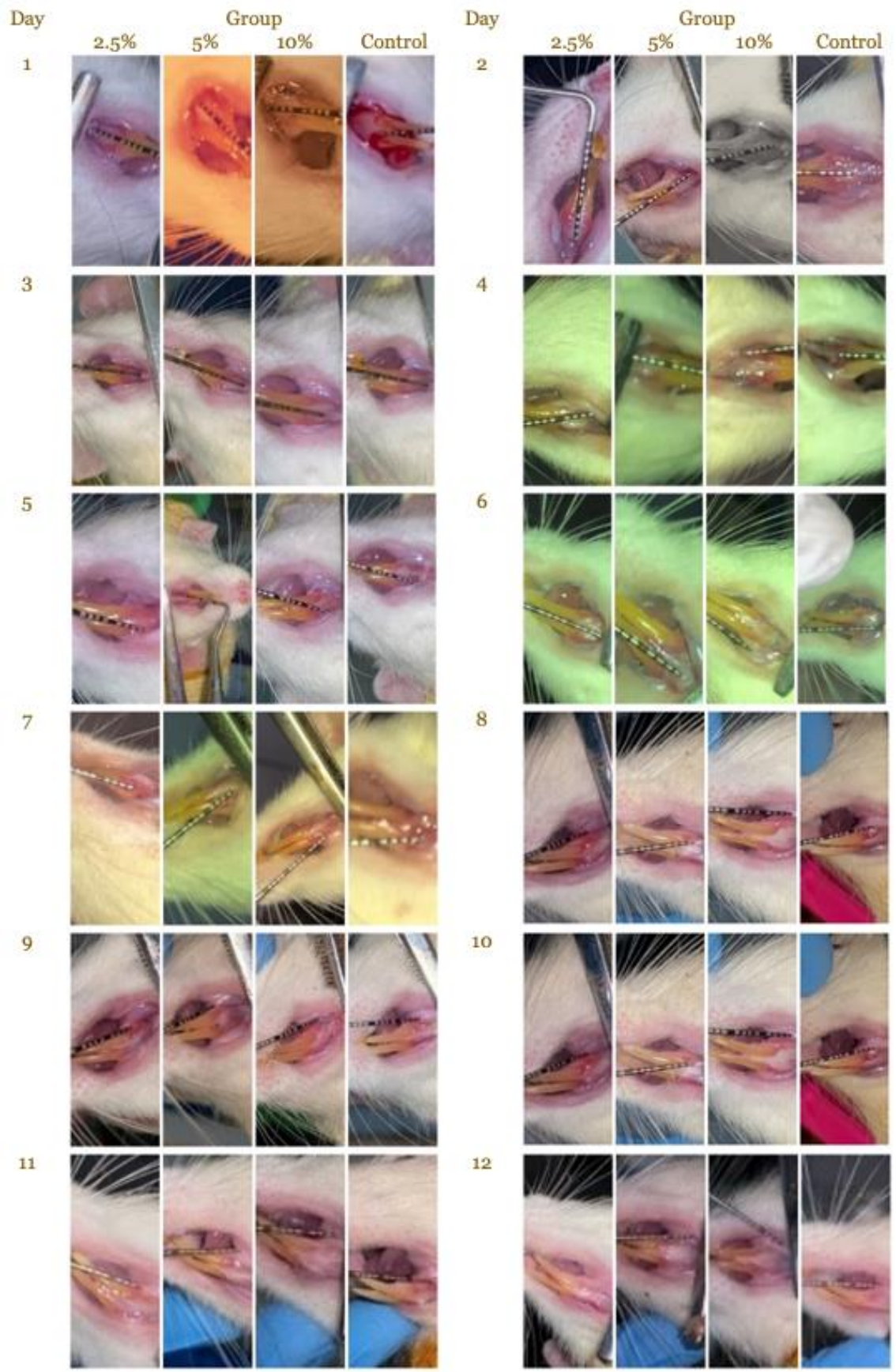

Figure 4. Comparison of wound healing closure from day 1 until day 12 between among groups of study.

Discussion

The *G. verrucosa* was collected from the Karawang region, West Java, Indonesia, contains alkaloids, steroids, flavonoids, and phenolic compounds. Several compounds have high percentage contents such as glycerol (36.81%), hexadecanoic acid (20.74%), and cholesterol (4.35%). These compounds are believed to play an important role in the wound-healing process. The steroids detected in the phytochemical test are suspected to be of the cholesterol type, as found in the GC-MS results.

In this study, an extract gel of *G. verrucosa* was produced and applied as a wound healer on experimental animals. Wound healing is a tightly regulated biological process in living organisms that aims to restore tissues after damage. The wound-healing process follows four overlapping phases: hemostasis, inflammation, proliferation, and remodeling [8]. In the hemostasis phase, the application of *G. verrucosa* extract gel to the mucosal wound in *R. norvegicus* showed reduced bleeding time. Adding *G. verrucosa* extract gel to the experimental group significantly reduced bleeding time compared to the control group. The groups with the shortest and longest bleeding times were treated with *G. verrucosa* extract gel at concentrations of 2.5%, 5%, and 10%. These results indicate that *G. verrucosa* extract gel can help accelerate wound healing in the hemostasis phase. At this stage, the optimal concentration of *G. verrucosa* extract gel for wound healing was 2.5%.

The GC-MS results indicate that glycerol is the most abundant compound detected in *G. verrucosa* samples. In this study, glycerol is believed to play an essential role in reducing the bleeding time of wounds. Glycerol has three hydroxyl groups, making it hygroscopic and water-soluble. It causes water to be drawn out from tissues and stored in the interstitial fluid and plasma by increasing blood plasma osmolality. Glycerol can absorb 3–4 times its weight in fluids. It moistens wounds and prevents exudates from drying and sticking to the skin. Glycerol has also been shown to be bacteriostatic, meaning it can prevent bacterial growth or reproduction [46]. Glycerol is also hypothesized to function as a glycerol transporter in wound healing, cell migration, and keratinocyte proliferation and differentiation [47]. Another important compound in the wound healing process contained in red algae extracts hexadecanoic acid, commonly known as palmitic acid. Hexadecanoic acid was the second most abundant compound in this study, accounting for 20.74%. This fatty acid has strong homeostatic control at the tissue level, which can be linked to its fundamental physiological role in ensuring the physical membrane properties and protein palmitoylation [48].

The next stage of wound healing is the proliferation stage. This stage is known to occur after six days from the time of bleeding. At this stage, there is an increase in the number of fibroblast cells. On the seventh day, the gel extract of *G. verrucosa* applied to the wound at concentrations of 0%, 2.5%, 5%, and 10% showed fibroblast cell counts of 9.8, 9.6, 10.6, and 7.8 cells, respectively. However, the increase in the number of fibroblast cells on the fourteenth day was not uniform. On the seventh day, the gel extract of *G. verrucosa* at concentrations of 0%, 2.5%, 5%, and 10% showed fibroblast cell counts of 11.9, 12.9, 13.9, and 14.9 cells, respectively. In the proliferation phase, research has reported that hexadecanoic acid can increase proline hydroxylation [32], thus increasing the number of fibroblast cells. After that, on the twenty-eighth day, there was a drastic decrease in fibroblast cells, where the *G. verrucosa* gel at concentrations of 0%, 2.5%, 5%, and 10% showed fibroblast cell counts of 1.3, 6.5, 7.3, and 7.3 cells, respectively. It indicates that the wound has passed the proliferation phase.

Based on the post hoc test presented in **Table 5**, it can be concluded that there was a significant difference in the mean number of fibroblasts between control and 10% on day 14, with a *p*-value of 0.007. However, the difference in the mean number of fibroblasts between the other treatment groups was insignificant. On day 28, the difference in the mean number of fibroblasts between control and 2.5%, 5%, and 10% was significant, while the difference in the mean number of fibroblasts between 2.5% and 5%, 2.5 and 10%, 5%, and 10% was not significant. The effect of *G. verrucosa* extract gel on the number of fibroblasts may be due to the presence of alkaloids in the red algae. Alkaloids are reported as one of the secondary metabolites that help in various phases of wound healing, including protein synthesis, wound contraction, cellular infiltration, neovascularization, and epithelialization [49]. In addition to alkaloids, steroid compounds also

aid in the process. Steroid content has been reported to affect the proliferation and differentiation of cells [50]. The type of steroid compound detected in this sample was cholesterol ($C_{27}H_{46}O$).

The duration of the wound healing process was also measured using the daily clinical examination method. The wound healing process of *R. norvegicus* was observed for 12 days, starting from days 1–12 after adding *G. verrucosa* extract gel at concentrations of 0%, 2.5%, 5%, and 10%. The wound closure development in the experimental group is presented in **Figure 4**. The control group generally experienced wound closure on day 11. Meanwhile, the experimental groups treated with *G. verrucosa* extract gel at 5% and 2.5% concentrations showed wound closure on day 10. The fastest wound closure occurred on day 9 in the experimental group treated with *G. verrucosa* extract gel at a concentration of 10%. The *G. verrucosa* extract is believed to accelerate wound closure and support molecular wound healing stages based on the results obtained. The compound content of fatty acyls is suspected of playing an important role in providing acidic conditions for the wound. Acid is used in wounds to limit infection and accelerate wound healing. The acid reduces the pH of the wound surface, making it an unfavorable environment for bacteria. Under acidic conditions, oxygen is released and distributed to damaged tissue. Oxygen in the wound tissue increases collagen synthesis and epithelialization. Furthermore, fibroblast activity will increase [51]. Oxygen is needed to train all wound healing processes for cell metabolism, especially energy synthesis through ATP, by stimulating angiogenesis, increasing keratinocyte differentiation, migration, and re-epithelialization, enhancing fibroblast proliferation and collagen formation, and allowing wound contraction, resulting in faster wound healing [52].

Conclusion

This study demonstrates that the extract of *G. verrucosa* taken from the Karawang region, West Java, Indonesia, contains compounds with the highest percentage of glycerol (36.81%), hexadecanoic acid (20.74%), and cholesterol (4.35%), which are believed to accelerate the wound healing process by supporting the hemostasis and proliferation phases. The decrease in the bleeding time of oral wounds in the experimental animals indicates the role of *G. verrucosa* extracts in the hemostasis phase. The optimal concentration of *G. verrucosa* extract in supporting the wound healing process is found to be 2.5%, which reduces the oral wound bleeding time to 33.98±2.66 seconds. In the proliferation phase, the highest fibroblast cell count is observed on day 14, where the experimental group receiving 10% *G. verrucosa* extract shows the highest fibroblast cell count. On day 28, the fibroblast cell count decreases, indicating the wound almost healed. This study shows that adding red algae gel extract to oral wounds can clinically accelerate wound healing. However, further studies, such as molecular investigations of each phase of the wound healing process (hemostasis, inflammation, proliferation, and remodeling), are needed to determine the involvement of the ethanol extract of red algae compounds in wound healing.

Ethics approval

This study was approved by the University of Syiah Kuala, Faculty of Dentistry, on 22 April 2020 in ethical clearance document number 186/KE/FKG/2020.

Competing interests

The authors declare no conflict of interest.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

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