Trop J Nat Prod Res, November 2020; 4(11):912-917 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Hemostatic and Wound Healing Effects of *Gracilaria verrucosa* Extract Gel in Albino Rats

Rachmi F. Hakim*, Fakhrurrazi Fakhrurrazi, Sri Rezeki, Liza M. Sari, Zuhra Marfirah

Faculty of Dentistry, Syiah Kuala University, Jl. Tgk. Hasan Krueng Kalee, Kopelma Darussalam, Kec Syiah Kuala, Banda Aceh City, Aceh 23111, Indonesia

ARTICLE INFO

Article history:
Received 17 August 2020
Revised 03 November 2020
Accepted 15 November 2020
Published online 30 November 2020

ABSTRACT

Wound healing in a hostile environment such as oral cavity requires more time. *Gracilaria verrucosa*, a species of the red algae has been reported to have hemostatic, wound healing, antibacterial and antioxidant potentials. The present study was designed to investigate the effect of *G. verrucosa* extract gel on oral incised wound healing in rats. *G. verrucosa* extract and gel were prepared. Phytochemical screening and antioxidant testing of the extract were carried out. Incision wound of 5 mm long in mandibula labial gingiva with a depth reaching the alveolar bone was made on ten albino rats and then divided into 2 groups. *G. verrucosa* extract gel (treatment) was applied topically twice a day for 14 days to the wound in the first group, while polyvinylpyrrolidone-sodium hyaluronate gel (control) was applied to the second group. The wounds were observed every day for 14 days. Bleeding time and wound healing potential of test gels were evaluated as well as histological examination of the gingival tissue of the experimental rats. The results showed that bleeding time for the treatment group was 70 ± 10 s, while the control group was 112.00 ± 8.37 s. The phytochemical screening indicated the presence of flavonoids, alkaloids, phenols, tannins, and saponins in *Gracilaria Verrucosa*. *G. verrucosa* extract gel significantly shortened the bleeding time and promoted faster wound healing process, which showed a better fibrillation toward incised oral wound healing in albino rats. Our findings indicated that *G. verrucosa* extract gel had both hemostatic and wound healing properties.

Keywords: Albino rats, Antioxidant, *Gracilaria verrucosa*, Hemostasis, Wound healing.

Introduction

Wound healing requires several processes that occur in a specific sequence. Intact hemostatic and inflammatory mechanisms are needed, to maintain equilibrium and give rise to newly formed tissues, trigger angiogenesis, and epithelialization, as well as collagen synthesis. All tissues follow an essentially identical pattern to promote healing with minimal scar formation. Wound healing is a protective function of the body that focuses on quick recovery where the regeneration process in a hostile environment takes more time. In particular, the oral cavity is a remarkable environment in which wound healing occurs in a warm oral fluid that contains millions of microorganisms. Oral mucosa wound healing comprises of a series of sequential responses that allow the closure of injury in this tissue. This process has critical importance to prevent the invasiocolln of microorganisms or other agents into tissues to prevent chronic inflammation from occurring since the oral mucosa is continually exposed to traumatic and infectious challenges.[1,2]

Wound healing consists of four main phases; hemostasis, inflammation, proliferation, and remodelling.[1] Hemostasis serves as the initial step of the healing process. Inflammation causes vasodilation and increases vascular permeability. If the body is injured, the initial response that arises is the control of bleeding through the process of clotting (coagulation) and hemostasis.[3]

*Corresponding author. E mail: rachmifananihakim@unsyiah.ac.id
Tel: (+62)8126900402

Citation: Hakim RF, Fakhrurrazi F, Rezeki S, Sari LM, Marfirah Z. Hemostatic and Wound Healing Effects of *Gracilaria verrucosa* Extract Gel in Albino Rats. Trop J Nat Prod Res. 2020; 4(11):912-917. doi.org/10.26538/tjnpr/v4i11.12

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

Disruption of hemostasis will trigger two important components to help blood fluids to remain in a balanced condition, namely blood coagulation and fibrinolysis.[4] The final phase of wound healing is the remodeling phase which is responsible for the development of new epithelium and the formation of scar tissue. In this phase, collagen remodeling occurs. Collagen is an important component in all phases of wound healing. It provides integrity and strength to tissues and is very important, especially in the proliferation and remodeling phases. Collagen also functions as a basis for intracellular matrix formation in the wound area.[5] If the wound healing process is disrupted by infection, dehiscence, hypoxia, or immune dysfunction, the secondary healing stage begins. During this stage, granulation tissue formation and epithelialization over this new tissue take place. This type of wound is more susceptible to infections and it can result in delayed wound healing.[6]

Gracilaria verrucosa is a species of the red algae (Rhodophyta), notable for its economic importance as an agarophyte and food for both humans and fish. The red algae scattered throughout the waters of the world, especially in the most abundant area of the equator, such as Indonesia.[7] Aceh Province is a maritime area, with marine resources that have not yet been optimally explored. Among the wealth of the sea that has the potential to be utilized in the biomedical field is seaweed, which is found in Indonesian waters, including Aceh. *G. verrucosa* is one of the many types of algae that grows in Indonesia and ranks the highest number of algae species that grow in Indonesian marine waters, which is about 452 species. The contents of *G. verrucosa* which include protein, sugar and fat, range from 14.4 - 23.8%, 32.4 - 49.3% and 0.6 - 3.6%, respectively. Red algae are also a good source of Ca, K, Mg, and Fe, which reinforces good nutritional value.[8] *G. verrucosa* contains polysaccharides which can be used to control capillary arteries and small veins by producing a hydrophilic effect, dehydrating blood, and concentrating its solid components, thereby increasing the formation of barriers. The algae have a unique composition because it originated from chloroplast and contain

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

fluoride from the cytoplasmic nucleus.[9] *G. verrucosa* is also known to contain flavonoids, alkaloids, saponins, triterpenoids, steroids and tannins that function as antioxidants and antibacterial.[10] Antioxidants have the potential to increase the synthesis of pro-collagen type 1 proteins.[11] Vitamin C in red algae also has been reported to have the potential of forming collagen.[12] This study was therefore aimed at investigating the potential of *Gracilaria verrucosa* extract gel as a topical hemostatic material in accelerating wound healing in albino rats.

Materials and Methods

Ethical approval

The research was an experimental laboratory type and used a post test-only control group. All procedures were approved by the Research Ethics Board of the Faculty of Dentistry, Syiah Kuala University for Animal Care and Use before the study was initiated. The Ethical clearance number was 186/KE/FKG/2020.

Animals

Ten healthy male albino rats (*Rattus norvegicus*) of 12-14 weeks old, weighing 200-300 g was obtained from the Faculty of Veterinary Medicine, Syiah Kuala University, Banda Aceh City, Indonesia. The albino rats were divided into 2 groups of 5 rats in each group.[13]

Source of Gracilaria verrucose

G. verrucose was obtained from Pulo Aceh in October 2019. Identification and authentication of the algae were done by Rahmad B MSi at the Pharmacology Laboratory Veterinary Faculty Universitas, Syiah Kuala, Indonesia, and voucher number B/2767/UN11.1.2/PT.01.05/2019 was allotted.

Preparation of Gracilaria verrucosa extract

Four (4) kg of *G. verrucosa* were washed with water, cut into small sizes and dried by aeration at room temperature (20 – 22^{o}C) for 8 days. The sample was then crushed using a blender until it became a fine powder. Maceration method was used to extract the red algae powder by dissolving it in 1 L of 96% ethanol and stirred every day for 2 days. Then, the red algae extract was filtered to obtain the residue. Thereafter, the filtrate was evaporated to dryness using a rotary evaporator at 40^{o}C under reduced pressure and a concentrated *G. verrucosa* extract was obtained.[14]

Phytochemical screening of Gracilaria verrucosa extract

Flavonoid test was carried out by mixing the red algae extract with magnesium powder, then 4-5 drops of concentrated HCl and 1-2 mL of 95% ethanol were added, and then shook vigorously. A colour change from red, yellow, to orange indicated the presence of flavonoids. Alkaloid test was done by adding *G. verrucosa* extract into a test tube and 0.5 mL of 2% HCl was also added and the solution was divided into two tubes. Two to three drops of Dragendorff's reagent, were added to tube 1 and 2-3 drops of Mayer reagent were added to tube 2. Formation of orange deposits in Tube 1 and yellowish deposits in Tube 2 indicated the presence of alkaloid. Saponin test was done by mixing the red algal extract and HCl in a test tube and the resulting solution was shaken vigorously in a vertical direction for 10 seconds. When a stable foam was formed, saponin was suspected to be present. The tannin test was carried out by boiling 0.5 g of *G. verrucosa* extract in 5 mL of distilled water, cooled and then filtered through a filter paper. The filtrate was added to 5 mL 0.1% $FeCl_3$ solution. Visible presence of tannin was indicated by the formation of a greenish-brown or dark blue colour. Phenol test was carried out by adding the extracts to 2 drops of 5% $FeCl_3$ solution. The formation of green or blue-green color indicated the presence of phenol compounds in the material. Steroid/triterpenoid test was carried out by dissolving 5 mg red algae in 2-3 ml of chloroform. Then Liebermann-Bouchard reagent (10 drops of acetic acid anhydride and 2-3 drops H_2SO_4) was added through the tube wall. A blue to green color indicated the presence of steroids, while brownish or violet rings at the boundary of the two solvents showed the presence of triterpenoids.[15]

Antioxidant activity testing of Gracilaria verrucosa extract

The 1,1-diphenyl-2-picrilhydrazyl (DPPH) method was used to determine the antioxidant activity of *G. verrucosa.* Five (5 mg) of the red algae was dissolved in 10 mL methanol, and then 1 mL of DPPH solution (0.4 mM) was added sequentially. The preparation was homogenized using a vortex mixer and incubated at 37°C in a dark room for 30 min. Thereafter, absorbance of the solution was measured using a spectrophotometer at the maximum absorption wavelength of DPPH (517 nm). Control absorbance was obtained by adding 7.9 mg DPPH solution into a 50 mL measuring flask, incubated at 37 °C and absorbance value was recorded.[16]

Preparation of Gracilaria verrucosa extract gel

The concentrated *G. verrucosa* extract was made into a gel by pouring it into a beaker together with distilled water and Na-CMC. The mixture was stirred until a homogeneous viscous mixture was obtained. Propylene glycol, glycerin, nipagin and TEA were added sequentially and stirred until it became homogenous. The red algal extract was dissolved in distilled water in another beaker at 50°C and mucilage Na-CMC was added and stirred for 15 min to form a gel mass. The gel was stored in vial bottles.[17]

Making incision wounds in albino rats' gingiva

The gingival labial portion under the two mandibular anterior teeth was the part chosen to be treated. All albino rats were anesthetized using a single intramuscular injection of 1-2 mg/kg xylazine hydrochloride and 10 mg/kg ketamine hydrochloride. After that, an incision was made using a scalpel and blade No. 11 along 5 mm with a depth reaching the bone.[13]

Examination of bleeding duration

The bleeding time was calculated by chronometer (Q & Q 1/100 Chrono) until blotting paper was no longer absorbing the blood. After the incision, *G. verrucosa* extract gel or polyvinylpyrrolidone-sodium hyaluronate gel was applied to the wound and the bleeding time was immediately determined. The duration of bleeding in each group was directly calculated by putting the blotting paper near the wound while not disrupting hemostasis.[18]

Evaluation of wound healing potential of Gracilaria verrucosa extract gel

All the incised albino rats were divided into 2 groups: To the first group, the red algal extract gel was applied (treatment group), while to the second group, polyvinylpyrrolidone-sodium hyaluronate gel was applied (control group). The topical application was done by applying 0.01 mL of extract gel to the incision wound using a 1 mL syringe twice a day, in the morning at 8.30 am and afternoon at 4.30 pm for a duration of 14 days. After the application, changes in wound were observed. The first day was taken as the formation of the wound and observed until reduction in the diameter of the wound by using a probe (UNC-15), which was a sign of healing.[13]

Observation of the reduction in wound diameter in the incised wounds in the experimental rats was carried out in each group every day using a periodontal probe (UNC-15). The wound healed if the diameter of the wound was 0 mm or complete closure of the wound occurred in the proliferation phase when the re-epithelialization process has occurred perfectly. At the end of the experiments (14th day post-wounding), the albino rats were euthanized and buried.[13] The final stage of this research was data collection based on the observations made in relation to how fast or effective the wound healing process took place in the experimental groups.

Histological analysis

Gingiva samples from the wound area were taken and kept in 4% formaldehyde in phosphate-buffered saline (PBS, pH 7.4) for 24 h. The samples were washed with PBS solution for 2 h, dehydrated using graded alcohol, clarified, and finally embedded into paraffin. A 6 µm-

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

thick section was cut with a motorized rotary microtome. Masson's trichrome was used to analyze collagen. The number of fibroblasts, collagen, and inflammatory cells was counted at the left, right, and center of the wound area. Stained tissue samples were observed using a polarizing microscope (Meiji Techno Microscopes Meiji Techno Co., Ltd.Japan), and Infinity software v6.4.1, 2013, Lumenera, Canada was used for all analysis.[19]

Statistical analysis

The data obtained were recorded and analyzed statistically using SPSS version 16 and hypothesis testing was performed with Mann Whitney test to assess the effect, by a comparison between red algae extract and polyvinylpirrolidone-sodium hyaluronate gels on wound healing in the experimental rats. The significance level was set at $P < 0.05$.

Results and Discussion

Wound healing is a complex process that restores injured tissue to normal conditions. It begins with the proliferation of fibroblast and deposition of collagen fibres, angiogenesis in granulation tissue, scar formation, wound contraction, and epithelialization.[20] Wound healing from injuries due to accidents or surgical interventions involves the activity of a complex network of blood cells, cytokines, and growth factors. There is increased metabolic demand for nutrients as a result of an increase in cellular activity, which emerged in the wound healing process.[21] In this study, incision was made in male albino rats' gingiva and the effect of *G. verrucosa* extract gel on bleeding time was first investigated. The results (Table 1) indicated that the average value of bleeding duration in treated albino rats was 70 seconds, while the control group was 112 seconds. This observation correlates with the study of Garcia-Manzano *et al*.[23] on normal hemorrhage male albino rats where bleeding duration ranged between 60-110 seconds for the control group, while the mean duration of bleeding in the *G. verrucosa* treatment group was 70 seconds. Consequently, the effect of *G. verrucosa* extract gel was observed on the reduction of bleeding duration time in the treatment group in relation to the control group. The algae contain polysaccharides that play a role as hemostatic agents.[22] The mechanisms of carbohydrates in *G. verrucosa* in the form of polysaccharides can be used to control capillary arteries and small veins by producing a hydrophilic effect, dehydrating blood, and concentrating its solid components, thereby increasing the formation of barriers.[9]

The results of the phytochemical screening revealed that red algal extract used in this study contained phytochemical compounds such as flavonoids, alkaloids, tannin, phenol saponins, and steroids as shown in Table 2. These act as antibacterial and antioxidants agents. The antibacterial agent reduced wound bacterial colonization and infection, to improve the healing process. Antibacterial agents shortened both the inflammatory period and healing time. Inflammation is the first response during the healing period as a defense mechanism of the tissue; a short duration in the inflammatory phase can shorten the healing process.[24] The antioxidant activity of *G. verrucosa* extract in this study was determined using the DPPH free radical test method by measuring absorbance values with a UV-Vis spectrophotometer at a wavelength of 517 nm (Table 3). IC_{50} value of the concentrated red algal extract has a value of 112.38 ppm (moderate). Antioxidant activity of *G. verrucosa* extract is related to the phenolic, tannin and flavonoid contents. The phenol level in *G. verrucosa* can be influenced by intrinsic and extrinsic factors. Intrinsic factors that can affect phenol levels are network productivity, life cycle stages, and age of red algae, while extrinsic factors are season, light intensity, depth, nutrition, geographic, and salinity.[25] The results of the DPPH assay of the red algal extract showed a moderate IC_{50} value which was associated with the presence of phenolic compounds in *G. verrucosa*.

Antioxidant activity in the test *G. verrucosa* may increase the synthesis of Type 1 pro-collagen proteins.[11] This could be observed in the histological image of the treatment group of *G. verrucosa* which showed a higher number of fibroblasts, compared to the polyvinylpirrolidone-sodium hyaluronate gel control group. Vitamins C and A contained in *G. verrucosa* also might play a role in the formation of collagen and epithelialization as a result of the ability of Vitamin C to synthesize collagen. More so, fibroblasts have a function of synthesizing collagen which is secreted into the extracellular space in form of pro-collagen. Furthermore, pro-collagen splits into terminal segments called tropo-collagen, which is then attached to another tropo-collagen molecules to form collagen filaments. These filaments are joined to form fibrils, which at the end forms collagen fibers. Vitamin C is needed by the hydroxylase enzyme as a cofactor. This hydroxylase enzyme will form hydroxyproline and hydroxylysine which are needed to form the collagen chain.[12] Vitamin A also plays an important role in increasing epithelialization. One of the hallmarks in the wound healing process is epithelialization that occurs in the proliferation phase.[26]

Polyvinylpirrolidone-sodium hyaluronate gel is commonly used for the treatment of oral ulcers, inflammation of the oral mucosa and prevention of oral microbial colonization.[27] In this study, wound healing in both the red algal extract and polyvinylpirrolidone-sodium hyaluronate gels showed that it occurred in the mandibular labial gingiva of albino rats which was marked by a reduction in wound size. The measurement of wound size was evaluated clinically by measuring the length of wound closure every day until it recuperated using a straight periodontal probe (UNC 15). The results of the average duration reduction of wound length of each group are presented in Table 4 and Figure 1. Both the treatment and control groups experienced a decrease in wound size on the 3rd day. Complete wound healing occurred on the 13th day in all the replicates of the treatment group, while complete wound healing occurred on the 14th day in the control group. Wound healing in the treatment group of *G. verrucosa* extract gel was faster compared with the polyvinylpirrolidone-sodium hyaluronate gel control group. The results of the Mann Whitney statistical test conducted in this research established the different cure rates for the two groups. The treatment group had an average cure rate of 12.60 days, while the control group had an average cure rate of 13.60 days. Mann Whitney test results showed a value of $p = 0.031$ ($p < 0.05$), so it could be interpreted that there was a significant difference in both test groups.

Histologically, wound healing of the gingival tissue of albino rats on the 14th day post application of *G. verucosa* extract or polyvinylpirrolidone-sodium hyaluronate gels are presented in Figure 2. In the *G. verrucosa* extract gel group, 216 fibrin, 20 collagen and no PMN were observed (Figure 2a), while 49 fibrin, 20 collagen and 5 PMN were recorded for the polyvinylpirrolidone-sodium hyaluronate gel group (Figure 2b). The absence of PMN on the 14th day in the *G. verrucosa* extract gel treatment group indicated that there was no more inflammatory phase in the wound healing process observed. Conversely, in the control group, the presence of 5 PMNs revealed that the inflammatory process was still ongoing. There have been many studies on wound healing process in albino rats that used herbs, such as Fetse *et al*.[28] that found extract of *Alstonia boonei* to have a significant effect on wound healing process, thereby proving the increased rate of wound contraction and reduction in the period of epithelialization. Tsala *et al*.[29] in their research showed the therapeutic potential of *Calotropis procera* on dermal wound healing, with a better collagen deposition and inflammatory reaction reduction.

Additionally, the study of Roodbordeii revealed an increase in wound healing activity and increased breaking strength after application of hydrogel containing total *F. vaillantii* extract.[30]

Our study examined the effect of *G. verrucosa* extract gel from the start of the wound healing process from hemostasis, inflammation, proliferation, to the initial remodeling process.

Table 1: Bleeding time in incised gingiva of albino rats treated with *Gracilaria verucosa* extract gel

Variable	Group	Mean ± SD
Bleeding Time	Control	112.0 ± 8.367seconds
	Gracilaria verucosa	70.00 ± 10.000seconds

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 2: Phytochemical constituents of *Gracilaria verrucosa* extract

Phytochemicals	Test result
Flavonoids	+
Alkaloids	+
Tannin	+
Phenol	+
Saponin	+
Steroids	+
Triterpenoid	-

+: Present; -: Absent

Table 3: Antioxidant activity of *Gracilaria verucosa* extract

Control (DPPH Abs.)	Conc. (ppm)	Abs. Sample	% Sample inhibitor	IC_{50}
0.92	25	0.882	4.13	112.383
0.92	50	0.655	28.80	112.383
0.92	100	0.573	41.63	112.383

It was demonstrated that *G. verrucosa* extract gel affected the oral incised wound healing process from the beginning of the process and also affected the clinical wound healing process indicated by a reduction in wound healing time. Histologically, fibrillation in the proliferation phase was better than the control group on the 14th day.

Table 4: Duration of wound length reduction in incised gingiva of albino rats treated with *Gracilaria verucosa* extract gel

Day	Wound length reduction (mm)									
	Treatment group (Rat replicate)					Control Group (Rat replicate)				
	1	2	3	4	5	1	2	3	4	5
1	5	5	5	5	5	5	5	5	5	5
2	5	5	5	5	5	5	5	5	5	5
3	4	4	4	4	4	4	4	4	4	4
4	3	3	3	4	3	3	3	4	4	3
5	3	3	3	3	3	3	3	4	3	3
6	3	3	3	3	3	3	3	3	3	3
7	3	2	2	2	2	2	3	3	2	3
8	2	2	2	2	2	2	2	2	2	2
9	2	2	2	2	1	2	2	2	2	2
10	2	1	2	2	1	2	2	2	2	1
11	1	1	2	2	1	1	2	2	2	1
12	1	0	1	1	0	1	1	1	1	1
13	0	0	0	0	0	0	1	1	0	1
14	0	0	0	0	0	0	0	0	0	0
Mean	12.60					13.60				

Treatment group: Treatment with red algal extract gel; Control group: Treatment with polyvinylpirrolidone-sodium hyaluronate gel

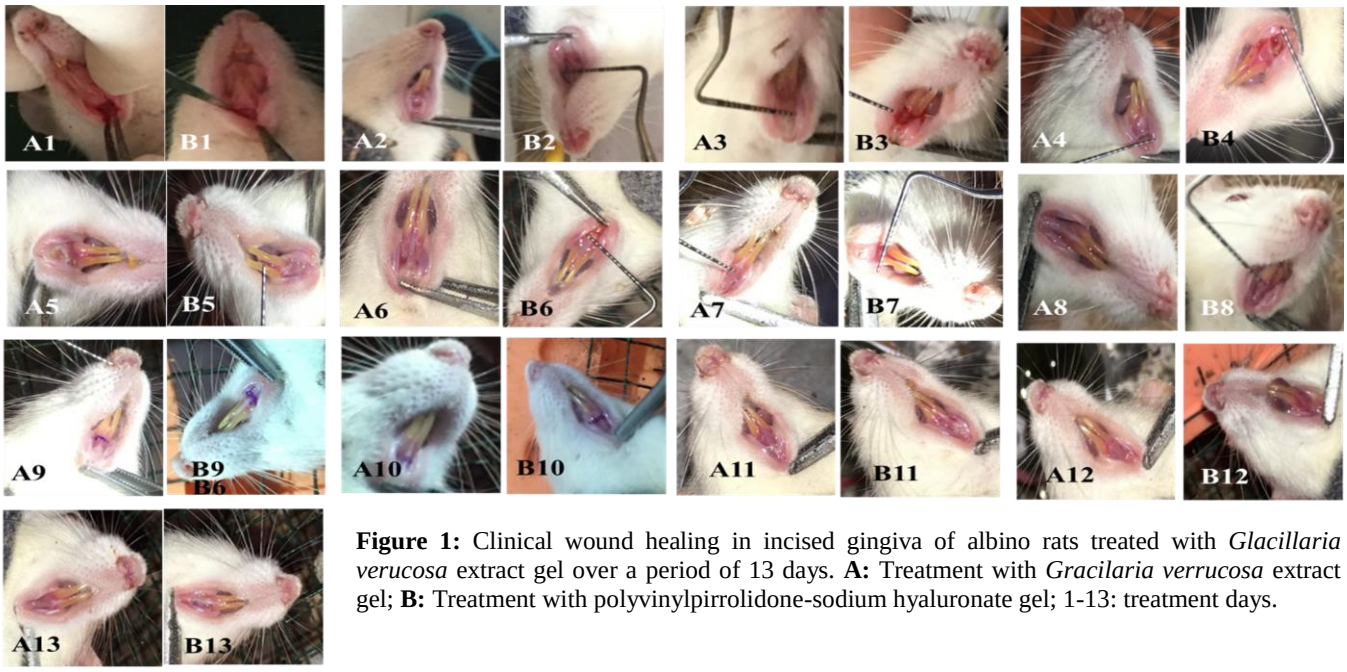

Figure 1: Clinical wound healing in incised gingiva of albino rats treated with *Glacillaria verucosa* extract gel over a period of 13 days. **A:** Treatment with *Gracilaria verrucosa* extract gel; **B:** Treatment with polyvinylpirrolidone-sodium hyaluronate gel; 1-13: treatment days.

Figure 2: Gel showing the presence and absence of PMN. **a:** *Gracilaria verucosa* extract gel without PMN; **b:** Polyvinyl-pirrolidone sodium hyaluronate gel showing 5 PMNs.

Conclusion

Gracilaria verrucosa extract gel provided a significant effect on reduction of bleeding time, faster wound healing process, clinically and histologically. At the 14th day, there were minimal inflammatory cells and more fibrillation in incised oral wound of albino rats. Our findings therefore, suggest a potential use of *G. verrucosa* in wound management.

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.

Acknowledgments

The authors wish to acknowledge the Universitas Syiah Kuala for providing funds for this research. Research grant Number: 270/UN11 /SPK/PNBP/ 2020 on March 17, 2020.

References

1. Politis C, Schoenaers J, Jacobs R, Agbaje JO. Wound healing problems in the mouth. Front Physiol. 2016; 7:507.
2. Bergmeier LA. Oral mucosa in health and disease. Springer Nature Switzerland. 2018. 77-90 p.
3. Beldon P. The basic science of wound healing. Surg. 2010; 28(9):409–412.
4. Motamedi MHK, Navi F, Koushki ES, Rouhipour R, Jafari SM. Hemostatic tampon to reduce bleeding following tooth extraction. Indian Red Crescent Med J. 2012; 14:386-388.
5. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. The J Int Med Res. 2009; 37(5):1528-1542.
6. Schultz GS. Mechanism of vascular disease principles of wound healing. London UK, University of Adelaide Press. 2001; 423-450 p.
7. Cian RE, Drago SR, Medina FSD, Augustin OM. Review proteins and carbohydrates from red seaweeds: evidence for beneficial effects on gut function and microbiota. Mar Drugs. 2015; 13:5358-5383.
8. Rodrigues D, Freitas AC, Pereira L, Teresa AP, Santos R, Marta W, Vasconcelos, Roriz M, Luís M, Rodríguez A, Gomes AMP, Duarte AC. Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal. Food Chem. 2015; 183:197-207.
9. Kumar MPS. Local hemostatic agents in the management of bleeding in oral surgery. Asian J Pharm Clin Res. 2016; 9(3):35-41.
10. Cirik S, Çetin Z, Ak I, Cirik S, Göksan T. Greenhouse cultivation of *Gracilaria verrucosa* (hudson) papenfuss and determination of chemical composition. J Fish Aqua Sci. 2010; 10:559-564.
11. Lee HY, Ghimeray AK, Yim JH, Chang MS. Antioxidant, collagen synthesis activity in vitro and clinical test on anti-wrinkle activity of formulated cream containing *veronica officinalis* extract. J Cosm Dermatol Sci Appl. 2015; 5:45-51.
12. De Phillipo NN, Aman ZS, Kennedy MI, Begley JP, Moatshe G, La Prade RF. Efficacy of vitamin c supplementation on collagen synthesis and oxidative stress after musculoskeletal injuries: A Systematic Review. The Orthoped J Sports Med. 2018; 6(10):1-9.
13. Hakim RF, Fakhrurrazi F and Dinni D. Effect of *Carica papaya* extract toward incised wound healing process in mice (*mus musculus*) clinically and histologically. Evid-Based Compl Altern Med. 2019; 2019:8306519.
14. Zhang Q, Lin L, Ye W. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018; 13(20):1-26.
15. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pac J Trop Biomed. 2014; 4 (Suppl 1):S359-S367.
16. Tenorio RPA, Murillo ÁJI, Campa CÁI. Antioxidant screening and phenolic content of ethanol extracts of selected Baja California Peninsula macroalgae. J Food Sci Technol. 2017; 54:422-429.
17. Buhus G, Popa M, Desbrieres J. Hydrogels based on carboxymethylcellulose and gelatin for inclusion and release of chloramphenicol. J Bioactive Compat Polym. 2009; 24:525-541.
18. Kaneda K, Kuroda S, Goto N, Sato D, Ohya K, Kasugai S. Is sodium alginate an alternative hemostatic material in the tooth extraction socket? J Oral Tiss Eng. 2008; 5(3):127-133.
19. Kim YS, Cho IH, Jeong MJ. Therapeutic effect of total ginseng saponin on skin wound healing. J Ginseng Res. 2011; 35(3):360-367.
20. Dhiyaaldeen SM, Alshawsh MA, Salama SM, Alwajeeh N SI, Al Batran R, Ismail S, Abdulla MA. Potential activity of 3-(2-chlorophenyl)-1-phenyl-propenonein accelerating wound healing in rats. Biomed Res Int. 2014; 2014: 792086.
21. Abood WN, Al Henhena, NA, Abood AN, Al Obaidi MMJ, Ismail S, Abdulla MA, Al Batran R. Wound-healing potential of the fruit extract of *Phaleria macrocarpa*. Bosnian J Basic Med Sci. 2015; 15(2):25-30.
22. Connan S, Delisle F, Deslandes E, Ar Gall E. Intra-thallus phlorotannin content and antioxidant activity in phaeophyceae of temperate waters. Bot Mar 2006; 49(1):39-46.
23. Polcz ME and Barbul A. The role of vitamin A in wound healing. Nutrition in clinical practice. Nutr Burn Injury 2019; 34(5):695-700.
24. Peng T. Biomaterials for haemorage control. Trends Biomater Artif Organs. 2010; 24(1):27-28.
25. Garcia MA, Gonzales L, Laven J, Lemini C, Rubio PC. Standardization of rat blood clotting tests with reagents used for humans. Proc West Phamacol Soc. 2001; 44:153-155.

26. Negut I, Grumezescu V, Grumezescu AM. Treatment strategies for infected wounds. Molecules. 2018; 23(9):2392.
27. Buchsel PC. Polyvinylpyrrolidone-sodium hyaluronate gel (Gelclair): a bioadherent oral gel for the treatment of oral mucositis and other painful oral lesions. Expert Opin Drug Metab Toxicol. 2008; 4(11):1449-1454.
28. Fetse JP, Kyekyeku JO, Dueve E, Mensah KB. Wound healing activity of total alkaloidal extract of the root bark of *Alstonia boonei* (apocynacea). Brit J Pharma Res. 2014; 4(23):2642-2652.
29. Tsala DE, Nga N, Thiery BNM, Bienvenue MT, Theophile D. Evaluation of the antioxidant activity and the healing action of the ethanol extract of *Calotropis procera* bark against surgical wounds. J Intercult Ethnopharmacol. 2015; 4(1):64-69.
30. Roodbordeii FD, Afshar M, Tabrizi FHA, Choopani S, Torkaman G, Moayer F, Salimi M. Topical hydrogel containing *Fumaria vaillantii Loisel*. extract enhances wound healing in rats. Compl Altern Med. 2019; 19(254):1-9.