

Effectiveness of *Sargassum sp.* ethanolic extract on traumatic ulcers healing in the labial mucosa of Wistar strain (*Rattus norvegicus*)

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ABSTRACT

Introduction: Oral ulcer lesions are one of the most common lesions in the oral cavity. The prevalence of traumatic ulcers is quite high compared to other oral lesions. Brown algae, *Sargassum sp.* is one of the algae contains flavonoids, vitamin A, vitamin C, iron, and calcium, which play a role in wound healing and has economic value. This study was aimed to determine the effectiveness of *Sargassum sp.* ethanolic extract on traumatic ulcers healing in the labial mucosa of Wistar strain (*Rattus norvegicus*). **Methods:** 28 Wistar strain (*Rattus norvegicus*) weighed 150-200 grams. The samples were divided into 4 groups (K1, K2, P1, P2); each group consisted of 7 mice. K1 group was administered with Hydroxy Propyl Methyl Cellulose (HPMC), K2 was administered with 0.2% hyaluronic acid gel, P1 was administered with 50% *Sargassum sp.* extract gel, and P2 was administered with 75% *Sargassum sp.* extract gel. In each group, the ulcer was made on the mice labial mucosa; then the ulcer diameter was measured and observed on day 1 and day 7. **Results:** The mean diameter \pm standard deviation of traumatic ulcer calculations were K1 (1.09 ± 0.40), K2 (1.81 ± 0.67), P1 (1.39 ± 0.43) and P2 (2.16 ± 0.49) respectively. One way ANOVA test results showed significant differences in K1, K2, P1, and P2 groups. **Conclusion:** Ethanol extract of *Sargassum sp.* is effective in accelerating the traumatic ulcers healing at the concentration of 75%.

Keywords: Traumatic ulcer, *Sargassum sp.*, wound healing.

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INTRODUCTION

Oral ulceration is one of the most common lesions encountered in the oral cavity.¹ The term ulcer is used when epithelial damage extends beyond the basement membrane. The aetiology of ulcers can vary, and the majority of which occur most often

are caused by mechanical trauma.² The primary purpose of wound healing after injury occurs is to restore continuity and function of the tissue.² In principle, traumatic ulcers will heal on the 7th day to 10th day if the cause is eliminated.³ The process of wound healing includes inflammation, proliferation, and remodeling.⁴ Traumatic ulcers

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are declared healed when judged by indicators of histopathological factors namely closure of inflammatory wounds and closing of the dermis which can be clinically seen by decreasing the diameter of the ulcer and without pain.⁵

Brown algae, *Sargassum sp.* is one of the algae that contains flavonoids, vitamin A, vitamin C, iron, calcium which play a role in wound healing and is economically valuable.⁶ *Sargassum sp.* has a large enough antioxidant content and plays a role in accelerating the inflammatory phase in which blood vessels and inflammatory cells such as Poly Morpho Nuclear (PMN), macrophages and lymphocytes have an essential role in killing and destroying antigens and producing antibodies called immunoglobulins.³

Sargassum sp. with higher doses up to 75 µg / g will increase cell viability, as evidenced by previous studies of *Sargassum sp.* with concentrations of 50% and 75% efficacious against collagen density in the healing process of traumatic ulcers, while administration at a dose of 25 µg / g has high toxicity, this is what makes the basis for researchers to find out the effectiveness of *Sargassum sp.* extracts. with concentrations of 50% and 75% of healing of traumatic ulcers.^{7,8}

Medical therapy in traumatic ulcers that are widely used today is aloclair with a hyaluronic acid content of 0.2% which functions as an anti-inflammatory so that it accelerates the healing process of traumatic ulcers. The current dose is used 3 times daily by applying it to the wound area.⁹ *Sargassum sp.* with concentrations of 50% and 75% are expected to be an alternative therapy for healing traumatic ulcers.

This study was aimed to determine the effectiveness of *Sargassum sp.* ethanolic extract on traumatic ulcers healing in the labial mucosa of Wistar strain (*Rattus norvegicus*). The results of this study were expected to provide valuable information about the effectiveness of *Sargassum sp.* on traumatic ulcers healing.

METHODS

The research conducted was true experimental laboratory research with post-test only control design. The experimental animal used was the Wistar strain (*Rattus norvegicus*). The period of the study until the completion of the reporting

was from July 2017-October 2017. The ethical appearance has obtained with registry number 012/KEPK/I/2018. The experimental animals were raised in the experimental animal unit of the Laboratory of Oral Biology, Faculty of Dentistry Hang Tuah University, Surabaya. *Sargassum sp.* extract was fabricated in the laboratory of the Faculty of Pharmacy Widya Mandala Catholic University, Surabaya.

The research instruments used were scales, spirit burner, amalgam stopper with a diameter of 1 mm, anatomical tweezers, chirurgis tweezers, freeze dryer, Erlenmeyer tube for *Sargassum sp.* gel, cement spatulas, handles and scalpels, masks and handschoen, hair polish (red, black, and blue), callipers for measuring the traumatic ulcers diameter, ovens for extracting, rotary evaporators for vaporising the filtrate, and animal pens sized 40 cm x 30 cm x 14 cm. The experimental unit in this study was the Wistar strain (*Rattus norvegicus*) obtained by simple random sampling.

The parameter observed in this study was the traumatic ulcer diameter. Twenty-eight rats were divided into four groups. K1 (negative control group), K2 (positive control group), P1 (50% *Sargassum sp.* extract gel group), and P2 (75% *Sargassum sp.* extract gel group). Traumatic ulcers were made using a burnisher, but in this study researchers used an amalgam stopper because the burnisher tip was too large so it was not possible to be used in making ulcers on the labial mucosa of the Wistar rat. Burnisher that has been heated for 1 minute above the spirit burner, then being touched to the labial mucosa with a depth of 1 mm and a diameter of 1 mm.¹⁰

The gel application was carried out according to the treatment group order K1, K2, P1, P2. K1 was given standard mouse feed, and 300 ml distilled water with topical Hydroxy Propyl Methyl Cellulose (HPMC) every day from the first day to the seventh day. K2 was given standard mouse feed, 300 ml distilled water with topical 0.2% hyaluronic acid gel every day from the first day to the seventh day. P1 was given standard mouse feed, 300 ml distilled water and also administered with 50% *Sargassum sp.* extract gel topically every day from the first day to the seventh day. P2 was given standard mouse feed, 300 ml distilled water and also administered with 75% *Sargassum sp.* extract gel topically every day

from the first day to the seventh day. The data scale used in this study was the ratio of sample

groups of more than two and unpaired so that the parametric statistical tests were performed.

RESULTS

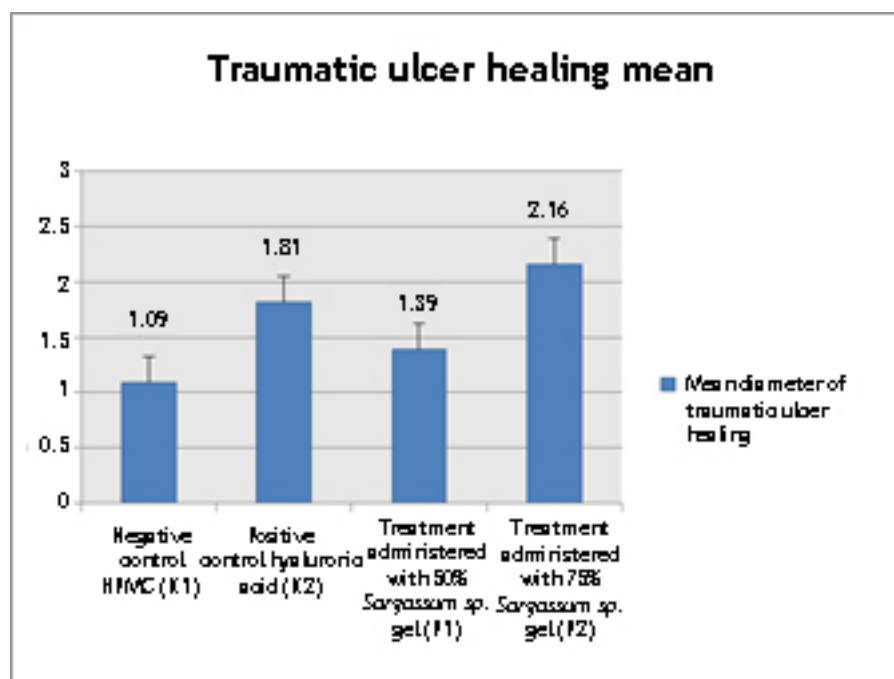


Figure 1. Mean diameter of traumatic ulcer healing

Figure 1 shows that the mean of traumatic ulcer diameter in the negative control group HPMC (K1), had the lowest results compared to other groups because HPMC which was a placebo did not give effect to accelerate healing, the second-lowest result was treatment group 1 which was given 50% *Sargassum sp.* (P1) then in the third place, the treatment group that was given 0.2% hyaluronic acid (K2) and the highest of all groups was the treatment group that was given 75% *Sargassum sp.* (P2), this is caused by 75% *Sargassum sp.* contains flavonoid, vitamin A, vitamin C, iron, calcium, and zinc to accelerate wound healing.

The normality test was conducted using the Shapiro-Wilk test because the number of samples was less than 50. The results of the normality test can be seen in Table 1.

Table 1. Shapiro-Wilk test results

Group	Sig
K1	0.742*
K2	0.622*
P1	0.052*
P2	0.993*

* = $p > 0.05$

Table 1 showed the significant value of each group $p > 0.05$ so that the data in this study can be stated normally distributed and can be continued with homogeneity test using the Levene's Test.

Table 2. Levene's test results

Levene statistic	Sig
1.381	0.308*

* = $p > 0.05$

Levene test results suggested that the data variation between groups in this study was homogeneous. The hypothesis test used was the one-way ANOVA (Analysis of Variance) test.

Table 3. One-way ANOVA test results

Variable	Sig
Ulcer diameter	0.010*

* = $p > 0.05$

Table 3 suggested a significance value of 0.010 ($p < 0.05$) which means that there was a difference in the effectiveness of *Sargassum sp.* extract gel in accelerating the healing of traumatic ulcers so that LSD test can be continued.

Table 4. LSD test results

Variable		Results
K1 (1.09)	K2	0.014*
	P1	0.709
	P2	0.034*
K2 (1.81)	K1	0.014*
	P1	0.006*
	P2	0.697
P1 (1.39)	K1	0.709
	K2	0.006*
	P2	0.015*
P2 (2.16)	K1	0.034*
	K2	0.697
	P1	0.015*

* = $p > 0.05$

LSD test results shown in Table 4 noted that there was a significant comparison of differences between K1 and K2 (0.014); K2 with P1 (0.006); K1 with P2 (0.034); and P1 with P2 (0.015) because the condition that there was a significant difference ($p < 0.05$).

DISCUSSION

The highest mean diameter was the administration of 75% *Sargassum sp.* Analytical test results using the one-way ANOVA test showed differences in effectiveness in accelerating the healing process of traumatic ulcers which were significant in each treatment group (Table 4). LSD test results (Table 4) showed that HPMC with 0.2% hyaluronic acid and HPMC with 75% *Sargassum sp.* showed significant difference. This is because HPMC is a gelling agent that is often used in the production of cosmetics and drugs, easily soluble in water and has low toxicity. So the content contained in HPMC cannot be used to cure traumatic ulcers because HPMC is a placebo in this study.¹¹ The group of rats given HPMC traumatic ulcers will heal by themselves without being affected by HPMC. This also applies to the significant differences in the HPMC and 75% *Sargassum sp.* group. HPMC which was a placebo does not have the effect of accelerating healing.⁷

A significant difference was also found between the 0.2% hyaluronic acid and 50% *Sargassum sp.* The main function of hyaluronic

acid accelerates wound healing.⁹ Hyaluronic acid is a part of the extracellular matrix and is the main glycosaminoglycan secreted during tissue repair. Hyaluronic acid also inhibits fibroblast proliferation. Hyaluronic acid can stimulate wound healing, migration, and mitosis of fibroblasts and epithelial cells and play an essential role in accelerating the process of wound closure,³ while 50% *Sargassum sp.* less effective for healing wounds. There was a significant difference between 50% *Sargassum sp.* and 75% *Sargassum sp.* This is caused by differences in the concentration of both 50% and 75%, where the content contained in 75% *Sargassum sp.* such as flavonoids, vitamins, iron, calcium, and zinc is more than 50% *Sargassum sp.*⁷

Some ingredients contained in *Sargassum sp.* are able to accelerate the healing of traumatic ulcers including flavonoids, vitamin A, vitamin C, calcium, iron and zinc. Flavonoids play a role in anti-inflammatory processes.¹² Flavonoids can function as potent toxins to kill bacteria and help speed up the inflammatory process by suppressing oxidative stress processes thereby decreasing lipid peroxidation. Decreased lipid peroxidation will cause an increase in collagen viability, thus accelerating the wound healing process.¹³ Wounds that were given extract of *Sargassum sp.* with flavonoid content will stimulate proliferation of fibroblasts, and activated fibroblasts will secrete collagen to form granulation tissue. The formation of a perfect granulation tissue will cover the wound surface.¹⁴

The content of vitamin A has the role of increasing the differentiation of epithelial cells and enhancing immunity.¹⁵ Vitamin A also has the function of accelerating the inflammatory phase to the proliferation phase by increasing macrophages to the wound area so that the phagocyte process can be immediately resolved.¹⁶ The content of vitamin C plays a role in stimulating the formation of collagen so that formation soon occurs new tissue.² Vitamin C is also needed in the remodelling phase which is to increase the strength of tissue stretching.¹⁷ The calcium content in *Sargassum sp.* can accelerate the process of reepithelialisation by increasing the proliferation of keratinocyte proliferation by characterised by increasing numbers of fibroblasts, collagen, and the formation of strong bonds between fibroblasts and extracellular matrix.¹⁸ Iron acts as an antioxidant

that supports the work of vitamin C so that vitamin C can work optimally in forming collagen.¹⁹ Zinc is needed to help the process of epithelialisation and proliferation of fibroblasts.

Not all subjects in the study showed uniformity in the healing process, and this could be due to the host factor (genetic factor) of each mouse that was different in terms of growth factor stimulation and cytokines responding to wound stimulation.²¹ Therefore it is recommended to do more research whether *Sargassum sp.* has the same effectiveness in the healing process of other lesions which also involve inflammation. In addition to the proven extract *Sargassum sp.* can accelerate healing of traumatic ulcers so that it can also be recommended the use of this extract as supportive therapy because drugs that have been circulating are synthetic drugs that tend to cause side effects.

CONCLUSION

Ethanol extract of *Sargassum sp.* is effective in accelerating the traumatic ulcers healing at the concentration of 75%.

REFERENCES

1. Pertiwi, NKFR. Pemberian Topikal Ekstrak Etanol Buah Adas (*Foeniculum Vulgare* Mill.) Konsentrasi 50% Lebih Meningkatkan Angiogenesis Dan Reepitelialisasi Daripada Povidone Iodine Untuk Penyembuhan Ulkus Traumatikus Mukosa Mulut Tikus Putih Jantan. Doctoral Dissertation, Universitas Udayana: Bali. 2015;11-20.
2. Wardani, Resti Puspita. Pengaruh Ekstrak Etanol Kulit Jeruk Nipis (*Citrus Aurantifolia* (Christm.) Swingle) Terhadap Penyembuhan Ulkus Traumatik Pada *Rattus Norvegicus* Strain Wistar. Diss. Universitas Muhammadiyah Surakarta. 2015;15.
3. Suwono AR, Soewondo IK, Revianti S. Efektivitas Gel Lendir Bekicot (*Achatina fulica*) Dalam Mempercepat Proses Penyembuhan Ulkus Traumatikus. Denta : Jurnal Kedokteran Gigi 2014;8(2):129-138.
4. Guo S, DiPietro LA. Critical Reviews in Oral Biology & Medicine: Factors Affecting Wound Healing. J Dent Res. 2010; 89(3): 219-229.
5. Jayadi, T. dan Krismi, A. Perbedaan Indikator-Indikator Penyembuhan Luka Tikus Wistar Non Diabetik Dan Diabetik Pada Pemberian Curcumin Topikal. Berkala Ilmiah Kedokteran Duta Wacana. 2015;1(1):11-27.
6. Ardiansyah MR. Analisis kadar flavonoid dari alga coklat (*Sargassum sp.* *Padina sp.*) Sebagai Obat Analgesik. Skripsi. Fakultas Kedokteran Gigi Universitas Hasanuddin, Makassar. 2017;10-11.
7. Karina A, Revianti S, Suwondo IK. Khasiat Ekstrak *Sargassum sp.* Terhadap Kepadatan Kolagen pada Proses Penyembuhan Ulkus Traumatikus. Denta : Jurnal Kedokteran Gigi 2014;8(1) :35-46.
8. Rustikawati, I. Efektivitas Ekstrak *Sargassum sp.* terhadap Differensiasi Leukosit Ikan Nila (*Oreochromis niloticus*) yang diinfeksi *Streptococcus iniae*. Jurnal Akuatika. 2012;3(2):125 - 134.
9. Kapoor, P., dan Sachdeva, S. Topical Hyaluronic Acid in The Management of Oral Ulcer. Indian J Dermatol. 2011;56(3):300-302.
10. Damaiyanti, DW. TGF B1 Expression on Traumatic Ulcer Healing Process Treated with Water Extract Gold Sea Cucumber. Research Article. Surabaya. 2016;195.
11. Sukmawan YP, Aryani R. Uji Aktivitas Penyembuhan Luka Formula Gel Ekstrak Etanol Daun Babandotan (*Ageratum conyzoides* L) Terhadap Tikus Wistar Jantan. Jurnal: Kesehatan Bakti Tunas Husada. 2016;16(1):88-95.
12. Muhammad, Rifqi Ardiansyah. Analisis Kadar Flavonoid Total dari Alga Coklat (*Sargassum sp.* dan *Padina sp.*) sebagai Obat Analgesik. Diss. 2017;23
13. Guyton, A.C., dan Hall, J.E. Buku Ajar Fisiologi Kedokteran. Edisi 12. Jakarta: EGC. 2014;125-127.
14. Palumpun EF, Wiraguna AAGP, Pangkahila W. Pemberian ekstrak daun sirih (*Piper betle*) secara topikal meningkatkan ketebalan epidermis, jumlah fibroblas, dan jumlah kolagen dalam proses penyembuhan luka pada tikus jantan galur Wistar (*Rattus norvegicus*), Jurnal e-Biomedik (eBm) 2017;5(1):5-7
15. Arissandi D. Pengaruh Basis Gel Poloxamer Dan Karbopol Terhadap Penyembuhan Luka Bakar Gel Ekstrak Etanol Umbi Wortel (*Daucus*

- carota L) Pada Kulit Punggung Kelinci. Skripsi. Fakultas Farmasi Universitas Muhammadiyah, Surakarta. 2009;24-28.
16. Negara WRFK, Ratnawati R, Dewi D. Pengaruh Perawatan Luka Bakar Derajat II Menggunakan Ekstrak Etanol Daun Sirih (*Piper betle* Linn.) Terhadap Peningkatan Ketebalan Jaringan Granulasi pada Tikus Putih (*Rattus norvegicus*) Jantan Galur. *Majalah Kesehatan FKUB*. 2014;1(2):86-93.
17. Ambiyani W. Pemberian Salep Ekstrak Daun Mengkudu (*Morinda Citrifolia* L) Meningkatkan Proses Regenerasi Jaringan Luka Pada Tikus Putih Galur Wistar (*Rattus Norvegicus*) Jantan. Skripsi. Fakultas Kedokteran Universitas Udayana, Bali. 2013;23-30
18. Asfa, Nur Wahidah. The Toxicity Of Brown Algae (*Sargassum Sp*) Extract To Mice (*Mus Musculus*). *Journal of Dentomaxillofacial Science*. 2016; 1(2): 115-109.
19. Novaritasari I. Uji Efektifitas Formulasi Gel Perasan Umbi Kentang (*Solanum Tuberosum* L.) Terhadap Lama Kesembuhan Luka Bakar Pada Kelinci (*Oryctolagus Cuniculus*) Putih Jantan. Skripsi. Universitas Ngundi Waluyo: Semarang. 2014;10-12
20. Sumanto J. Hubungan Antara Asupan Vitamin C dan Zinc dengan Proses Penyembuhan Luka Pasien Pasca Caesarean Section di Instalasi Rawat Jalan di Rumah Sakit PKU Muhammadiyah Surakarta. Skripsi. Universitas Muhammadiyah Surakarta: Surakarta. 2016;4-6
21. Suhartono, Michelle, and Sularsih Sularsih. "Perbedaan Pengaruh Aplikasi Gel Kombinasi Kitosan Berat Molekul Tinggi dan Rendah dengan Ekstrak Lidah Buaya (*Aloe vera*) Terhadap Kepadatan Kolagen pada Proses Penyembuhan Ulkus Traumatikus." *DENTA Jurnal Kedokteran Gigi*. 2018;12(1):60