

Formulation and Evaluation of Transdermal Films from Green Betel Leaves (*Piper betle* L.), Red Seaweed (*Eucheuma cottonii*), and Black Marlin Fish Skin (*Istiompax indica*) as Topical Therapy for Abrasions

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Abstract

Bali is renowned for its traditional healing practices known as Bali Usada, which utilize natural herbs and spices. The Balinese people have long used betel leaves and seaweed as traditional remedies for wound healing. Untreated wounds can lead to complications like infections and chronic inflammation, potentially causing scars. While conventional medical drugs can have side effects and often target specific issues, this research aims to develop a new practical therapy that promotes wound healing and tissue repair through collagen. Contains various useful secondary metabolites from green betel leaves (*Piper betle* L.), black marlin skin (*Istiompax indica*), and seaweed (*Eucheuma cottonii*) such as alkaloids, flavonoids, phenolics, saponins, tannins, collagen, and others. These molecules facilitate wound healing through antibacterial, antioxidant, and anti-inflammatory activities. These three materials are made in the form of thin filaments applied by placing them on the wound. The filaments disappear after a short time and absorbed into the skin locally to provide minimal side effects. The film evaluated using antibacterial tests, DPPH tests, organoleptic and thickness tests, dissolution time tests, and wound development and histopathology tests. The results state the film is able to provide better wound healing and inhibit the growth of *S. aureus* bacteria, has a number of antioxidant activities, has no odor, is not sticky, is not thick, and dissolves quickly. Wound healing occurred better by administering film with formulation 1, namely a ratio of betel leaf extract, black marlin collagen extract, and red seaweed extract 1:1:1. Overall, this study provides a preliminary information of the application of betel leaves, red seaweed and black marlin collagen as an alternative potential to be applied for wound healing.

Keywords: Abrasions, black marlin skin, betel leaf, seaweed, transdermal film

INTRODUCTION

The problem of wounds has not stopped being a concern for researchers lately. With the highest prevalence of around 70.9%, abrasions are greatly underestimated with people's understanding that wounds only occur on the skin (1). In fact, abrasions can become complications that cause health problems such as infection, bleeding, ulcers, keloids, and traumatic scars. Recommendations for treating abrasions are limited to cleaning the wound, applying antibacterial ointment, and using anti-tetanus drugs (2). In these recommendations, there are no aspects of scar treatment that are very likely caused by abrasions considering the

characteristics of the wound which is easily visible and very likely to leave scars (3).

Scar treatment is based on the need for several ingredients such as antioxidants or vitamin C which are important in increasing collagen in the skin, creating new blood vessels, and helping the absorption of iron to overcome bleeding. In addition, collagen is also needed which plays a role in inflammation, angiogenesis, and wound repair (4). The combination of antioxidants and collagen is also believed to be able to ward off free radicals and heal wounds better (5).

Bali is known as one of the provinces in Indonesia whose traditions

are still well preserved. The use of natural ingredients as medicine is inseparable from the touch of tradition that is still maintained and applied by the Balinese. Betel as a leaf that is often used has been scientifically proven to have many health benefits. With its antibacterial content, betel can be used in healing wounds, especially those contaminated with bacteria so that it can prevent infection (6). Seaweed is also often consumed through *rujak* which in fact has antioxidant content (7). Based on this, researchers have made a breakthrough in wound medicine that has a film or thin layer preparation form with antioxidant content from seaweed, antibacterial from betel leaves, and additional collagen from fish skin which is useful in treating healing and repairing post-wound tissue, especially abrasions. This preparation form has the advantage of being easy to carry anywhere and practical to use, making it easier to treat wounds (8). This therapy is expected to not only repair post-wound tissue and prevent infection, but can also minimize inflammation. This really needs to be researched in order to improve the quality of life of someone who experiences wounds and create good healing of abrasions without scars.

METHOD

This research has been declared ethically feasible by the Health Research Ethics Commission of the Faculty of Medicine and Health Science, Warmadewa University with number 409/Unwar/FKIK/EC-KEPK/III/2024 dated March 28, 2024. This research is an experimental research conducted from April to July at the Research and Experimental Animal Laboratory of the Faculty of Medicine and Health Sciences, Warmadewa University.

Extraction Process

Green betel leaves were obtained from the Badung Traditional Market in Denpasar City. Seaweed was obtained from Farmers in Serangan Village,

Denpasar City. Black Marlin Fish Skin waste obtained from the Kedonganan Fish Market, Badung Regency. Betel leaf and seaweed extracts were made using the extraction method with 96% ethanol with a ratio of 1:5 which was left for 2x24 hours. Furthermore, it was filtered and put into a rotary evaporator to obtain an extract with a concentration of 100% without any solvent. The next extract can be made according to the concentration variation by mixing it with 96% ethanol (9).

Black marlin skin collagen extraction begins by first cleaning the skin from scales. The meat is discarded to leave only the skin. The fish skin is cut into smaller pieces and soaked in 0.1M NaOH solution for 24 hours. After that, it is neutralized with distilled water until the pH level reaches seven. Second, soak the skin in CH₃COOH or 0.5 M acetic acid for 3 days. After that, filter it with a cloth and soak it in 0.9M NaCl for 1 day. Once done, the fish skin is centrifuged at 3500 rpm for 20 minutes. Discard the water and soak the skin again in CH₃COOH with a concentration of 0.1M for 12 hours. Finally, soak it in 0.9M NaCl for one day. After everything is done, discard all the water and dry it. Drying can be done using the oven method or freeze dryer (10).

Antibacterial Test of Betel Leaf Extract

Antibacterial tests were carried out using the disc diffusion method. After the petri dish is filled with Mueller Hinton Agar growing media and *Staphylococcus aureus* bacteria are grown, the disc paper that has been soaked in the extract will be placed on the petri dish (6). After 24 hours, the horizontal and vertical diameters are measured. The results will be interpreted weak with diameter <5 mm, 5 to 10 mm being categorized as moderate, 10 to 20 mm being interpreted as strong, and above 20 mm being interpreted as very strong. The data will be entered into SPSS version 29 software to carry out a comparative test with One-Way ANOVA with a

significance limit of 0.05 using a positive control of 1% Ampicillin and a negative control of 96% ethanol.

DPPH Test of Seaweed Extract

The DPPH test is carried out in several stages, namely, making a 2,2-diphenyl-1-picrylhydrazyl(DPPH) solution, determining the maximum DPPH wavelength, examining the concentration of seaweed extract antioxidants. First, the DPPH solution was made with DPPH powder weighed as much as 0.007 grams and dissolved with 50 mL of ethanol, homogenized with vortex until dissolved. Next, 1 mL of DPPH solution was taken and ethanol was added until it had a volume of 5 mL and left for 30 minutes. Second, 1 mL of DPPH solution was taken with a pipette and 5 mL of ethanol solution was added and left for 30 minutes in a dark place then the absorbance was measured at a wavelength of 517 nm. Third, seaweed

extracts with various concentrations (20 ppm, 40 ppm, 60 ppm, 80 ppm, 100ppm) were left for 30 minutes then the maximum wavelength was read at 517 nm, with a comparison solution of vitamin C concentration (2ppm, 4ppm, 6ppm, 8ppm, and 10 ppm) with the same treatment (11).

Film Formulation

This film was made by the solvent casting method. Previously in a petri dish, betel leaf extract, seaweed extract, and collagen from fish skin will be dissolved in distilled water according to the specified ratio. Hydroxypropyl methyl cellulose (HPMC) is then dissolved in distilled water that already contains nipagin and the three ingredients. Polyethylene glycol (PEG) 400 is added and stirred again. Then it is ovened at 50°C for 1 day or left at room temperature for 3 days (8). The amount of ingredients for the film formulation follows table 1.

Material	Formulation 1	Formulation 2
HPMC E5	400mg	400mg
Nipagin	6mg	6mg
Betel Leaf Extract	21mg	32mg
Seaweed Extract	43mg	38mg
Fish Collagen Extract	43mg	38mg
Aquadest	6g	6g
PEG 400	40mg	40mg

Organoleptic Test, Thickness, and Dissolution Time

Organoleptic test is done by looking at the color and odor. A good film will have organoleptic test results that are clear, odorless, smooth, non-sticky, and transparent. Meanwhile, the thickness is measured with a micrometer at five points or five repetitions. The optimum thickness ranges from 0.05mm to 0.1mm (8,12). Dissolution time or disintegration time is measured by dissolving the biofilm in a petri dish that has been filled with 2mL of distilled water. The results will be considered good if the disintegration time is below 1 minute (12). The thickness and

dissolution time variables will then be analyzed statistically with SPSS version 29 software through a comparative test, namely the Independent T Test with a significance limit of 0.05.

Wound Development and Skin Histopathology Test

The experimental animals or mice were acclimatized first for seven days by being given adequate food and drink to eliminate all other factors. After that, the mice will be shaved on their backs. Subcutaneous injection of lidocaine at a dose of 0.1 cc/100 g was performed before the wound was made in an area of approximately 2 cm (13). The application

of the film was carried out by following the recommendations of the Ministry of Health, namely the wound was cleaned with running water first. A humid environment will be created, at which time the film will be attached. A humid environment is needed to dissolve the film. The application was carried out with a time span of 24 hours (2,8). The wound was observed by visual assessment. The assessment was carried out based on the wound closure process. Wound repair was evaluated every day (13–15). Skin histopathology was carried out by looking at two parameters. First, epithelialization and second, inflammatory cell infiltration. Two mice from each group will have their skin taken for histopathology with hematoxylin-eosin staining (15).

RESULT

Antibacterial Test of Betel Leaf Extract Test of Inhibition Zone Diameter

Group	Mean (mm) $\pm$ SD	p-value
Negative Control (N)	0 $\pm$ 0	
Betel Leaf extract (E)	15.8425 $\pm$ 0.88447	< 0.001
Positive Control (P)	12.2725 $\pm$ 0.14683	

Antibacterial test of betel leaf extract against *Staphylococcus aureus* bacteria has an average inhibition of 15.8425 mm $\pm$ 0.88447 which can be seen in Figure 1. The diameter of this inhibition zone is larger than the positive control using 1% ampicillin. Overall, there is a significant difference based on the inhibition zone diameter data with a p-value of <0.001 (16). Based on the LSD post hoc test to determine the significance of the difference in the average diameter of the inhibition zone of each group, the comparison of each group with other groups has a significant difference with a p-value of <0.001 (16). This can be interpreted as betel leaf extract is able to inhibit the growth of *staphylococcus aureus* bacteria even better than the standard antibiotic used, namely ampicillin.

Table 2 Results of the One-Way ANOVA

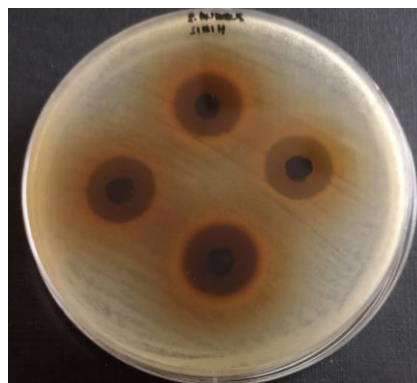


Figure 1 *Staphylococcus aureus* Bacterial Inhibition Zone with Betel Leaf Extract
DPPH Test of Seaweed Extract

The antioxidant activity test was carried out with DPPH, namely by observing the color change in the DPPH solution and performing spectrophotometry with a maximum wave of 517nm. Through testing, it was found that the seaweed extract showed moderate antioxidant activity which was evaluated

from the color change from purple to yellow even at the smallest concentration (5 ppm) (17). The IC₅₀ value describes the antioxidant strength, namely the concentration of the sample solution needed to inhibit 50% of free radicals. A low value indicates increasingly strong antioxidant activity. Based on the results

of the study and calculations using a linear regression equation from the sample concentration curve to antioxidant activity, the IC₅₀ value of the green seaweed extract was 108,630 ppm which was classified as moderate (17). The results showed that *E.cottonii* seaweed extract has strong antioxidant ability to reduce free radicals.

Organoleptic Test, Thickness, and Dissolution Time

Organoleptic tests were carried out to evaluate the color, odor, and characteristics of the film. Both formulations were able to produce films that had a clear color even though there were some blackish patterns as in Figure 2. Both film formulations did not produce a pungent odor, and were not sticky.

The thickness was measured with a micrometer five times. The optimal thickness for transdermal films ranges from 0.05 to 0.1mm (12). Formulation 2 had an average thickness of 0.108mm $\pm$ 0.01924 and was thicker than formulation 1 with a thickness of 0.076mm $\pm$ 0.02408. Based on the Independent T test, there was a significant difference between the

thickness of the two films with a p-value of 0.049 (16).

The dissolution time test of the Film preparation gave various dissolution time results, ranging from 80-120 seconds which were obtained by placing the Film on a petri dish containing 2 mL of distilled water. Formulation 1 has an average dissolution time of 85.8 seconds $\pm$ 25.9171 which is faster than formulation 2 with an average dissolution time of 114.2 seconds $\pm$ 4.9444. Overall, based on the Independent T test, there is a significant difference between the dissolution times of formulations 1 and 2 of 0.042 (16).

Wound Development Test and Skin Histopathology

Wound development tests are carried out every day. This will evaluate the condition of the wound after 24 hours of treatment. Based on Figure 3, the untreated group showed a picture of poor wound healing, namely the wound became dry and did not close. The group treated with formulation 1 film showed a picture of a wound with healing that almost closed perfectly on the fifth day, as did the group treated with formulation 2 film.

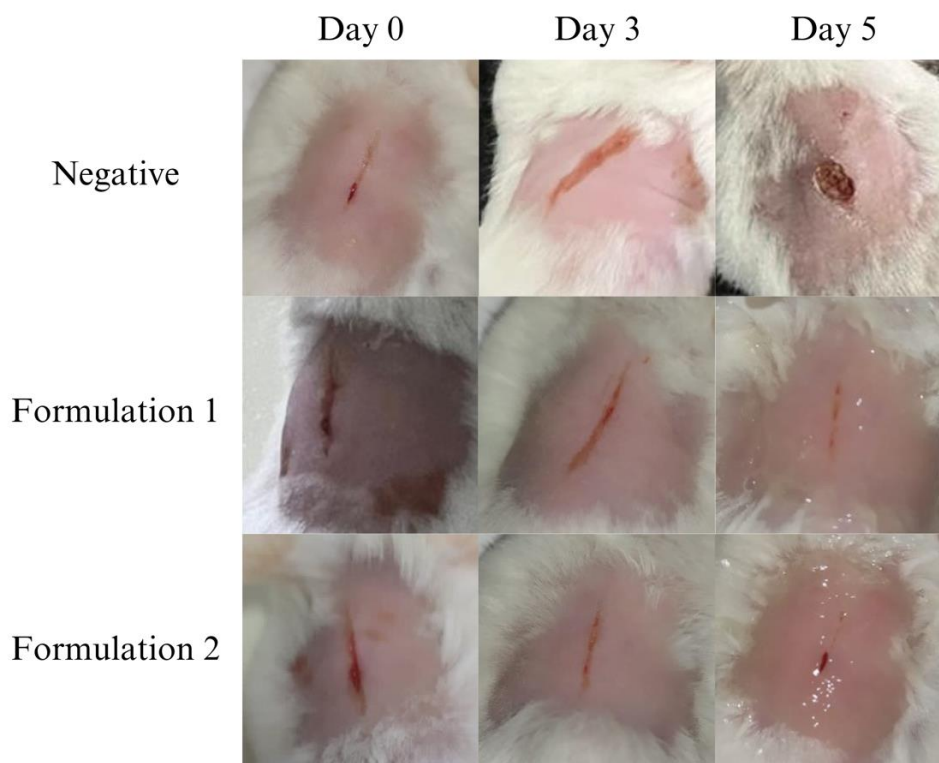


Figure 8. Wound Development Test Results. (A) Wound on the First Day Without Treatment, (B) Wound on the Third Day Without Treatment, (C) Wound on the Fifth Day Without Treatment, (D) Wound on the First Day with Formulation 1 Film, (E), Wound on the Third Day with Formulation 1 Film, (F) Wound on the Fifth Day with Formulation 1 Film, (G) Wound on the First Day with Formulation 2 Film, (H), Wound on the Third Day with Formulation 2 Film, (I) Wound on the Fifth Day with Formulation 2 Film.

Histopathology was performed to assess the wound healing process microscopically by evaluating epithelialization or the addition of epithelial cells and infiltration of inflammatory cells such as neutrophils, macrophages, lymphocytes, and others. Figure 4 shows the histology of the skin of the group without treatment in the form of inflammatory cell infiltration and epithelium that is not completely intact.

The histology of the skin treated with formulation 1 film shows a picture of massive epithelialization and no inflammatory cell infiltration. Wounds treated with formulation 2 film showed epithelialization that had occurred without inflammatory cell infiltration with non-intact extracellular matrix. Epithelialization occurred more in the group treated with formulation 1 film compared to formulation 2.

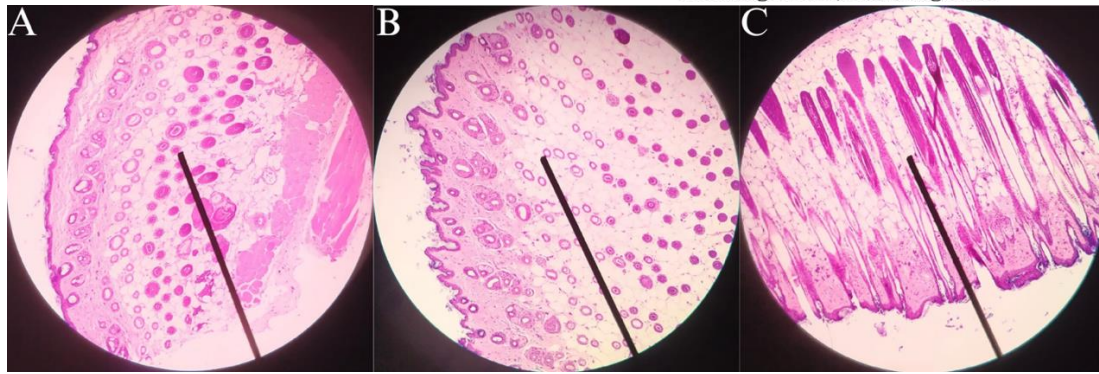


Figure 9. Histology of Mice Skin (A) Formulation Film Therapy 2, (B) Formulation Film Therapy 1, (C) Without Treatment.

DISCUSSION

Based on the results obtained, the ethanol extract of betel leaves is classified as having a strong inhibitory power against the test bacteria, namely *Staphylococcus aureus*. The results of previous research tests showed similar results where the ethanol extract of green betel leaves provided an inhibition zone of 23.3 mm against *S. aureus* at a concentration of 80% (6). The contents of the ethanol extract of betel leaves can effectively inhibit the growth of the test bacteria. *S. aureus*, which is a Gram-positive bacteria that only consists of several layers of peptidoglycan, causes this bacteria to be easily denatured by the compounds contained in the green betel leaf extract. Several chemical compounds contained in green betel leaves and have antibacterial activity are phenol, kavikol, alkaloids, flavonoids, and terpenoids (18). Phenol and its derivative compounds contained in the ethanol extract of betel leaves limit various bacterial developments. Phenolic compounds can denature bacterial proteins and damage bacterial membranes (19). In addition, at high concentrations, phenol can also damage the cytoplasmic membrane completely (19). Kavikol compounds have much stronger antibacterial activity compared to phenol. The way kavikol compounds work is by denaturing cell proteins which causes microorganisms to be killed (20). Alkaloid compounds work

by disrupting the peptidoglycan components in bacterial cells which are used for bacterial survival in hypotonic environments. Flavonoid compounds can disrupt the function of bacterial cell walls by forming complexes with extracellular proteins and inhibiting bacterial motility. Damage to lipids and amino acids which are components of bacterial cell walls will react with the alcohol groups of flavonoid compounds, causing the compounds to seep into the bacterial cell nucleus. Terpenoid compounds can inhibit bacterial growth by disrupting the process of membrane or cell wall formation. This will cause the bacterial cell wall to not form perfectly so that it is easier to destroy (21).

Previous research revealed that the IC₅₀ value of *E. cottonii* extract was 31,440 ppm which was classified as very strong. The study gave different results from this study, where the antioxidants of this study were only classified as moderate. The study used extracting antioxidants from seaweed with the soxhletation method which uses heating so that it is able to free antioxidants that are insoluble at room temperature (22). Other studies have given some different results. Crude seaweed extract has a very strong antioxidant content, but with 10x and 100x dilution, the antioxidant content is reduced to weak and non-existent (23). It was also revealed that drying with UV light can affect the antioxidant results obtained (17). Other studies using seaweed in the

Pajukukang Bantaeng area gave weak antioxidant results (17). It was stated that differences in location affect the antioxidant content (17,22).

The film is made through a drying and heating process using an oven. This causes all the water contained as a solvent to evaporate so that the film becomes denser. This explains why the film is not sticky. The film will become sticky again if it is exposed to water during application. The film can still be said to be clear even though it has a slight yellow to brownish color given by betel leaves and seaweed that go through a drying process to heating (24). However, the film appears brownish due to the content of betel leaf extract, namely essential oils that cannot be completely homogeneous with the solvent used, namely distilled water (25).

Thickness is an important aspect in evaluating the film. Thickness is influenced by the amount of material used and will affect the dissolution time later. There is no definite rule regarding the optimal thickness (12). If the film can provide the effect as designed as a wound treatment and remains economical to carry around and the dissolution time remains fast, the thickness can be said to be optimal. Other studies have made films with a thickness of 0.098 which is said to be the best (12). Similar to this study which has a thickness not far from previous studies.

Wound development tests are basically intended to determine the wound closure process and see whether complications such as wounds experiencing secondary infections occur or not. Wound development involves the process of tissue recovery. Tissue recovery occurs in the form of structural repair efforts from a wound or injury. There are two end results of the tissue recovery process, namely regeneration and scar tissue formation (26). Tissue regeneration occurs when damaged cells are replaced so that the structural and

functional tissue will return to their pre-injury condition. Scar tissue is formed when cells in the injury are unable to regenerate due to severe damage or there is no effort to repair the healing process. The structure of the tissue can be maintained with the presence of scar tissue, but not for its function (26).

The tissue regeneration process involves healthy cells around the injury and growth factors. Not only that, the extracellular matrix that functions as a space for cells to carry out their activities also has an equally important role. ECM Plays a role in regulating cell proliferation, providing a basic tissue framework for the tissue regeneration process, to creating a micro-environment of tissue such as a connection between the layers above and below (26). This extracellular matrix has several components such as collagen, elastin, proteoglycan, hyaluronate, and glycoprotein (27,28). Collagen is the largest component of the extracellular matrix in the wound healing process (26). Lack of collagen will cause imperfect wound healing, but excess collagen can also cause problems such as the formation of scar tissue. Therefore, collagen is one of the crucial aspects in the tissue regeneration process.

Wound healing occurred better in the group that received film therapy compared to not receiving treatment, of course because of the contents of the film such as collagen, antibacterial substances, and antioxidants that help the wound healing process. Giving collagen is considered to be the right step to accelerate the wound healing process as evidenced by Figure 8. However, giving collagen must be accompanied by giving vitamin C which will make collagen stronger (26). Vitamin C is one of the antioxidants contained in betel leaves and seaweed (23,29). Previous studies have used collagen as a wound healing material and stated that collagen is one of the wound therapies that will provide a good

wound healing process (4). This study is in line with this study. The wound closure process that can be evaluated visually in order to evaluate the wound healing process appears equally good in the group treated with formulation one or two films.

Infection is one of the important factors that can inhibit the process of tissue regeneration after injury (26). The presence of secondary infection can prolong the inflammatory process that has occurred previously and will expand the area that has been injured or scarred. The concept of preventing secondary infection in wound care in general is to drain the wound on the skin in running water (30). In fact, previous research has said that rinsing with running water on the wound is one of the important things that supports wound healing (30). The administration of antiseptic fluids such as povidone iodine is also often done to prevent microbes from growing in the injured area which can cause secondary infections (31). The addition of antibacterial substances contained in betel leaves and antioxidants from seaweed aims to prevent this secondary infection. Both of these contents can inhibit or kill the growth of microbes so that secondary infections do not occur and the wound healing process takes place properly. Evaluation of secondary infection is done by looking at signs of inflammation, namely rubor, dolor, calor, tumor, and leisa functia around the wound (26). Based on this, both groups treated with formulation 1 and formulation 2 provided equally good prevention of secondary infections. The use of antibacterial and antioxidant substances to treat skin infections by previous studies stated that healing occurred better than the control group that used only several types of antibiotics (32).

Antioxidants have been reported to provide better outcomes in the wound healing process. This is related to thicker epithelialization through microscopic

evaluation. Even the healing that occurs almost touches the perfect number. Antioxidants, one of which is quercetin contained in green betel leaves, are able to control pro-inflammatory cytokines and induce anti-inflammatory cytokines, induce angiogenesis, to fibroblast and collagen proliferation which ends with better wound healing (5). There are several mechanisms of epithelialization induction that are influenced by antioxidants. Increased production of anti-inflammatory cytokines such as IL-10 to increased angiogenesis factors, namely Vascular Endothelial Growth Factor or VEGF, are important keys to the wound healing process (33). Inhibition of pro-inflammatory cytokines such as Tumor Necrosis Factor- α to inhibition of scar tissue formation factors, namely Transforming Growth Factor- α -1 also play a role (33).

Inflammatory cell infiltration is evaluated through histological images of neutrophils, macrophages, lymphocytes, and others according to their characteristics such as color, granules, to their nuclei (29,31). According to previous research, antioxidant administration reduces the incidence of inflammatory cell infiltration (34). Other studies have shown that antioxidant content such as flavonoids, tannins, saponins, and alkaloids contained in green betel leaves and seaweed can inhibit pro-inflammatory cytokines such as IL-1, IL-6, TNF- α , and others that play a major role in decreasing (35,36).

CONCLUSION

A film with antioxidant, antibacterial, and collagen content made from green betel leaf extract, seaweed extract, and black marlin fish extract can be used as topical therapy for abrasions. This is supported by several results stating that wound healing occurs better in the group treated with the film. Betel leaf extract has strong antibacterial activity.

Seaweed extract has moderate antioxidant content. Organoleptic and thickness tests show that the film is clear, odorless, non-sticky, and has good thickness. The time required to dissolve is quite good. The use of the film accelerates wound healing and closure with a better appearance. The epithelialization process occurs more and inflammatory cell infiltration is less in the group using the film with antioxidant, antibacterial, and collagen content.

However, this study still has several shortcomings such as the absence of a standard protocol related to dissolution time testing. Measurement of antibacterial, antioxidant, and collagen content contained in the film in this study was not carried out because there was no such testing protocol. The inhibition zone diameter test using the final film product against *Staphylococcus aureus* bacteria should also be carried out. Several additional tests are needed such as phytochemical tests to determine the antioxidant content in the seaweed samples and quantitative wound development tests by measuring the width of the wound.

ACKNOWLEDGEMENTS

This research has been financially funded by the Faculty of Medicine and Health Sciences, Warmadewa University, under grant number registration number 1437/Unwar/FKIK/PD-13/VIII/2023 for Putu Khrisna Dharma Jaya. Authors would like also to thanks also to all parties involved in the implementation of this research.

BIBLIOGRAPHY

1. Wintoko R, Yadika ADN. Manajemen Terkini Perawatan Luka. *JK Unila*. 2020;4(2):183–9.
2. Kementerian Kesehatan Republik Indonesia. Luka Lecet. 2022. Available from:

- https://yankes.kemkes.go.id/view_artikel/873/luka-lecet
3. Ratu RNDC, Pamuttu A, Bension JB. Karakteristik dan Pola Luka Korban Kecelakaan Lalu Lintas di Rumah Sakit Bhayangkara Ambon Periode 2014-2017. *Moluscca Medica*. 2021;14(1):63–9.
4. Steiner SSM, Roy S, Sen CK. Collagen in Wound Healing. *Bioengineering*. 2021 May 1;8:1–15.
5. Sanz IM comino, Franco MD lopez, Castro B, Hidalgo PL pancarbo. The role of antioxidants on wound healing: A review of the current evidence. *J Clin Med*. 2021 Aug 2;10:1–22.
6. Alydrus NL, Khofifah N. Efektifitas Antibakteri Ekstrak Daun Sirih Hijau (*Piper Betle L*) Terhadap *Staphylococcus Aureus*. *Indonesian health Journal*. 2022;1(1):56–61.
7. Dwimayasanti R. Rumput Laut: Antioksidan Alami Penangkal Radikal Bebas. *Oseana*. 2018;2:13–23.
8. Zubaydah WOS, Sahumena MH. Fast Dissolving Oral Film Salbutamol Sulfat dengan Menggunakan Polimer HPMC. *Indonesian Journal of Chemometrics and Pharmaceutical Analysis*. 2021;1(3):133–42. Available from: www.journal.ugm.ac.id/v3/IJCPA
9. Azzahra F, Hayati M. Uji Aktivitas Ekstral Daun Pegagan (*Centella asiatica (L). Urb*) Terhadap Pertumbuhan *Streptococcus mutans*. *Jurnal B-Dent*. 2018;5(1):9–19.
10. Kolanus MJP, Hadinoto S, Idrus S. Karakterisasi Kolagen Larut Asam dari Kulit Ikan Tuna (*Thunnus albacores*.) dengan Metode Hidroekstraksi. *Jurnal Riset*

11. Prasetyo E, Kharomah NZW, Rahayu TP. Uji Aktivitas Antioksidan Menggunakan Metode DPPH (2,2-difenil-1-pikrilhidrazil) Terhadap Ekstrak Etanol Kulit Buah Durian (*Durio zibethinnus L.*) dari Desa Alasmalang Kabupaten Banyumas. *Jurnal Pharmascience*. 2021;08(01):75–82. Available from: <https://ppjp.ulm.ac.id/journal/index.php/pharmascience>
12. Febriyenti, Syofyan, Jessica A, Assofiah NH, Novira DA. Formulasi Sediaan Orally Dissolving Film (ODF) Propanolol dan Dispersi Padat Kuersetin. [Palembang]: Universitas Andalasa; 2020.
13. Fazri ATSK. Pengaruh Pemberian Ekstrak Kosambi (*Schleichera oleosa*) Terhadap Percepatan Penyembuhan Luka Eksisi pada Mencit (*Mus musculus*). [Malang]: Universitas Islam Negeri Maulana Malik Ibrahim; 2019.
14. Nasution MFW, Yenita. Uji Efektivitas Madu Dibandingkan dengan Povidone Iodine Terhadap penyembuhan Luka Sayat pada Mencit (*Mus musculus*). *JIMKI*. 2020;8(3):47–53.
15. Sari LN, Kanedi M, Yulianty, Ernawati E. Efektivitas Ekstrak Etanol Daun Knikir (*Cosmos caudatus Kunth*) Terhadap Penyembuhan Luka Sayat pada Mencit (*Mus musculus L.*). *Jurnal Tadris Biologi*. 2019;10(2):109–20. Available from: <http://ejournal.radenintan.ac.id/index.php/biosfer/index>
16. Dahlan MS. Statistik Untuk Kedokteran dan Kesehatan. Sixth Edition. Epidemiologi Indonesia; 2014.
17. Husna S, Nurlaida, Utami HH, Najmah. Komponen Fitokimia dan Aktivitas Antioksidan Rumput Laut *Eucheuma cottonii* di Peraian desa Pajukukang Bantaeng. *Jurnal Crystal: Publikasi Penelitian Kimia dan Terapannya*. 2023;5(2):9–15.
18. Suarantika F, patricia VM, Rahmah H. Optimasi Proses Ekstraksi Daun Sirih Hijau (*Piper betle L.*) yang Memiliki Aktivitas Antioksidan Berdasarkan Penggunaan secara Empiris. *Jurnal Ilmiah Medicamento*. 2023;9(1):16–21.
19. Ogana AS, Sulistyowati E, Widyaningrum I. Penghambatan Pertumbuhan *Staphylococcus aureus* dan *Eschericia coli* oleh Kombinasi Ekstrak Metanol Rimpang *Alpinia purpurata* dan *Zingiber officinale*. *Jurnal Bio Komplementer Medicine*. 2021;8(2):1–8.
20. Sadih H, Cahyadi A, Windria S. Kajian Potensi Daun Sirih Hijau (*Piper bitle L.*) Sebagai Antibakteri. *Jurnal Sains Veteriner*. 2022;40(2):128–38.
21. Sujono H, Rizal S, Purbayasa S, Jarmansyah. Uji Aktivitas Antibakteri Minyak Atsiri Daun Sirih Hijau (*Piper betle L.*) terhadap Bakteri *Streptococcus pyogenes* dan *Staphylococcus aureus*. *J Kartika Kimia*. 2019;2(1):30–6.
22. Rakhmawati IAI, Sukarno, Sitanggang AB. Antioxidant Activity of DPPH From Seaweed Extract Using Meta-Analysis Study. *J Pengolah Has Perikan Indones*. 2023 Dec 18;26(3):520–34.
23. Prasetyo H, Sasongko AS, Fahira D, Ayuningsih T. Skrining Fitokimia Dan Uji Aktivitas Antioksidan Secara Kualitatif pada Ekstrak Rumput Laut *Eucheumma*

- cottonii. Jurnal Kemaritiman: Indonesian Journal of Maritime. 2023;4(1):25–34.
24. Amrih D, Syarifah AN, Gusta M, Budiarti P, Safitri A, Nugraha ISA. Pengaruh Pemanasan Terhadap Perubahan Warna Pada Pangan. *Journal of Innovative Food Technology and Agricultural Product*. 2023 Feb 3;01(01):1–4.
25. Gunawan D, Kurniaty R. Pemanfaatan Minyak Atsiri Daun Sirih (*Piper Betle Linn*) Sebagai Anti Nyamuk. *Journal of Pharmaceutical and Health Research*. 2021 Jun 30;2(2):46–9.
26. Kumar V, Abbas AK, Aster JC. Robbins & Cotran Pathologic Basis of Disease. Tenth. Philadelphia: Elsevier; 2021.
27. Karamanos NK, Theocharis AD, Piperigkou Z, Manou D, Passi A, Skandalis SS, et al. A Guide to The Composition and Functions of The Extracellular Matrix. *FEBS Journal*. 2021 Dec 1;288(24):6850–912.
28. Zhang W, Liu Y, Zhang H. Extracellular Matrix: An Important Regulator of Cell Functions and Skeletal Muscle Development. *Cell Biosci*. 2021 Dec 1;11(65):1–11.
29. Nuryahya MF, Afiani N, Soelistyoningsih D. Perbedaan Efektivitas Ekstrak Daun Sirih Hijau dan Merah pada Perawatan Luka Derajat II. *Media Husada Journal of Nursing Science*. 2020;1(1):1–10. Available from: <https://ojs.widyagamahusada.ac.id>
30. Masi GMN, Katuuk ME, Rotty MPF. Efektivitas Air Mineral untuk Pencucian Luka Kronis. *Jurnal Keperawatan*. 2023;11(1):81–5.
31. Altoé LS, Alves RS, Sarandy MM, Morais-Santos M, Novaes RD, Gonçalves RV. Does Antibiotic Use Accelerate or Retard Cutaneous Repair? A Systematic Review in Animal Models. *PLoS One*. 2019 Oct 1;14(10):1–22.
32. Elnahas RA, Elwakil BH, Elshewemi SS, Olama ZA. Egyptian Olea europaea Leaves Bioactive Extract: Antibacterial and Wound Healing Activity in Normal and Diabetic Rats. *J Tradit Complement Med*. 2021 Sep 1;11(5):427–34.
33. Suratmini PA, Wardani IGA, Suena NMDS. Potensi Tanaman Obat Terhadap Epitelisasi dalam Penyembuhan Luka Bakar. *Jurnal Integrasi Obat Tradisional*. 2021;1(1):9–16. Available from: <https://usadha.unmas.ac.id>
34. Latif AC. Gambaran Histopatologi Ginjal yang Diinduksi Gentamisin dan Pemberian Tepung Buah Jambu Biji Merah (*Psidium guajava L.*) pada Tikus Putih (*Rattus norvegicus*). In: *Seminar Nasional Cendekiawan ke 5 Tahun 2019*. 2019. p. 1.71.1-1.71.7.
35. Hamidy MY, Rangkuti IF, Masdar H, Adila AF, Baihaqi F. Pengaruh Ekstrak Buah Bakau terhadap Infiltrasi Sel Radang pada Hati Tikus yang Diberi Diet Tinggi Lemak. *Unram Medical Journal*. 2023 Dec 31;12(4):407–10.
36. Ansari MY, Ahmad N, Haqqi TM. Oxidative Stress and Inflammation in Osteoarthritis Pathogenesis: Role of Polyphenols. *Biomedicine and Pharmacotherapy*. 2020 Sep 1;129:1–9.