Effectivity of sea grape extract (*Caulerpa Sp.*) against wound cure on the angle of wistar white rats (*Rattus norvegicus*) lips induced by *Staphylococcus aureus* and *Candida albicans*

Efektivitas ekstrak anggur laut (*Caulerpa Sp*.) terhadap penyembuhan luka pada sudut bibir tikus putih wistar (*Rattus norvegicus*) yang diinduksi *Staphylococcus aureus* dan *Candida albicans*

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ABSTRACT

Introduction: Apart from fish, other marine products that can be processed are seaweed. Sea grape (*Caulerpa Sp.*) is one of the seaweeds, which contains active ingredients, namely flavonoid, saponin, carotenoid, phenol, alkaloid and tannin which are useful as antioxidant, antibacterial, antidiabetic and cytotoxin, antinociceptive and anti-inflammatory. **Objective**: To determine the effect of sea grape extract towards wound healing on the corners of the lips of white wistar rats (*Rattus norvegicus*) induced by *S.aureus* and *C.albicans*, and to compare them with the antimicrobial gentamicin and the antifungal miconazole. **Methods**: Pure experimental research with posttest only control group design; the wound of 24 male wistar white rats divided into: group 1 induced by *S.aureus* and then given sea grape extract, group 2 induced by *C.albicans* then smeared with sea grape extract, group 3 induced by *S.aureus* then given gentamicin, and group 4 was induced by *C.albicans* and then given miconazole. The length of the wound was periodically measured as an indicator of the healing process. Data were analyzed using SPSS version 22.0. **Results**: The effect on each group were tested using Friedman, showed significant in each group (p = 0.001). Different test with Mann Whitney group 1 and group 3 (antibacterial control) showed significantly different results on testing days 1-5; group 2 and group 4 (antifungal control) also showed significantly different results on days 1-4; groups 1 and 2 showed insignificant results. **Conclusion**: Sea grape extract had a significant effect on wound healing in the lip corners of male wistar rats induced by *S.aureus* and *C.albicans*. Sea grape extract had the same effect with positive antibacterial and antifungal control.
Keywords: *Caulerpa Sp.,* healing, wound, corner lips

ABSTRAK

Pendahuluan: Selain ikan, hasil laut lainnya yang dapat diolah adalah rumput laut. Anggur laut (*Caulerpa Sp.*) merupakan salah satu rumput laut yang memiliki kandungan bahan aktif, yaitu flavonoid, saponin, karotenoid, fenol, alkaloid dan tanin yang bermanfaat sebagai antioksidan, antibakteri, antidiabetik dan sitotoksin, *antinociceptive* dan anti-inflamasi. **Tujuan**: untuk mengetahui pengaruh ekstrak anggur laut (EAL) terhadap penyembuhan luka pada sudut bibir tikus wistar putih (*Rattus norvegicus*) yang diinduksi *S.aureus* dan *C.albicans*, serta membandingkannya dengan antimikroba gentamisin dan antijamur miconazole. **Metode**: Penelitian eksperimen murni dengan rancangan *posttest only control group*; pembuatan luka pada sudut bibir terhadap 24 ekor tikus putih jantan wistar yang dibagi atas kelompok 1 diinduksi *S.aureus* kemudian diberi EAL, kelompok 2 diinduksi *C.albicans* kemudian diolesi EAL, kelompok 3 diinduksi *S.aureus* kemudian diberi gentamisin, dan kelompok 4 diinduksi *C. albicans* kemudian diberi miconazole. Panjang luka secara periodik diukur sebagai indikator proses penyembuhan. Data dianalisis menggunakan program SPSS versi 22.0. **Hasil**: Uji pengaruh pada masing-masing kelompok dengan menggunakan Friedman, menunjukkan signifikan pada masing-masing kelompok (p=0,001). Uji beda dengan Mann Whitney kelompok 1 dengan kelompok 3 (kontrol antibakteri) menunjukkan hasil yang berbeda nyata pada pengujian hari 1-5; kelompok 2 dan kelompok 4 (kontrol antijamur) juga menunjukkan hasil yang berbeda nyata pada hari 1-4; kelompok 1 dan 2 menunjukkan hasil yang tidak signifikan. **Simpulan**: EAL berpengaruh nyata terhadap penyembuhan luka pada sudut bibir tikus putih wistar jantan yang diinduksi *S.aureus* dan *C.albicans*; dan memiliki efek yang sama dengan kontrol antibakteri dan antijamur positif.
Kata kunci: *Caulerpa Sp*., penyembuhan, luka, sudut bibir
Received: 10 January 2021 Accepted:15 February 2022 Published: 1 April 2022

INTRODUCTION

Indonesia's geographical position is very unique, being in the tropics in a cross position between two continents with two oceans so that from this geographical condition Indonesia has abundant natural wealth. Indonesia has biological resources that are available on land and in abundant waters. Indonesia is known as a country with a sea area of more than 70% marine products are generally fish, other alternative sea products that can be processed are seaweed.[2] Seaweed is one of the leading commodities with potential as food. Seaweed is rich in fiber, vitamins and minerals and is a source of natural

DOI 10.35856/mdj.v11i1.506

antioxidants that are easily available in quite abundant quantities. One type of seaweed that is potential is sea grapes (*Caulerpa sp.*).[1] Sea grape has a high enough vitamin content, including vitamin A, vitamin C, iron, iodine and calcium. Since 2007, Japanese have been known to consume a lot of sea grapes in fresh form. Sea grape is also believed to have properties for treatment and beauty.[2]

According to the study, sea grape extract can be useful as an antibiotic, anti-inflammatory, antioxidant, antifungal and antimicrobial which has been specifically investigated for its inhibition and shows good results.[3]

Angular cheilitis, which has another name for angular cheilosis, commissural cheilitis, angular stomatitis, or perleche, is an oral lesion characterized by fissures, redness or desquamation of the corners of the mouth accompanied by pain, dryness, burning and sometimes accompanied by itching.[4]

Wounds on the corners of the lips can affect all ages, not limited to certain age groups, children and adolescents can be affected by angular cheilitis regardless of gender.[5] *Angular cheilitis* is caused by various factors such as nutritional deficiencies, mechanical trauma, infection, and allergies.[6] *Angular cheilitis* is most often caused by infection and includes organisms such as *Can-dida, S.aureus, and β-hemolytic streptococci bacteria*.[7]

Treatment of *angular cheilitis* is very dependent on the cause. The main causes are fungal and bacterial infections. Provision of antifungal ointments such as miconazole 2% topically applied in the corner of the mouth is often used as a healing material.[8] Based on this explanation, the author would like to examine the effects of sea grape extract which is used as an alternative for wound healing in the corners of the lips of male white rats induced by *S.aureus* and *C.albicans*.

METHODS

This type of research will be conducted is a true experimental laboratory with a posttest only control group design, conducted at the Phytochemical Laboratory of the Faculty of Pharmacy, and the Entymology Laboratory of the Faculty of Medicine, Hasanuddin University in October 2019. The samples were white male wistar strain rats which were divided into four groups, namely group 1 induced by *S.aureus* and then sea grape extract was applied, group 2 induced by *C.albicans* then applied sea grape extract, group 3 induced by *S.aureus* then gentamicin were applied as positive antibacterial control, and group 4 induced by *C.albicans* then miconazole were applied as antifungal positive control.

Inclusion criteria were wistar healthy strain male white rats, have 150-250 g body weight, and they were 2-3 months old. The exclusion criteria were white mice that cannot adapt, such as not eating; white mice that are sick or die during the adaptation process.

As for the ingredients used are white rat food and drinks were sea grape extract, ethanol 96%, *S.aureus* inoculum, *C.albicans* inoculum, cotton, 2% miconazole ointment, gentamicin ointment 0.1%, lidocaine. The tools used were tweezers, runes, rulers, white mouse cages, jars, rounded ose, brushes, *handscoens* and masks.

Sea grape extract is a sea herbal plant where all the parts are taken and then extracted. Sea grapes chosen are sea grapes that are green, and still intact form obtained from Takalar Regency. The dried plants were extracted by maceration method using 96% ethanol solvent. After maceration for 3 days the filtering was then evaporated using a *rotary evaporator* to produce a thick extract.

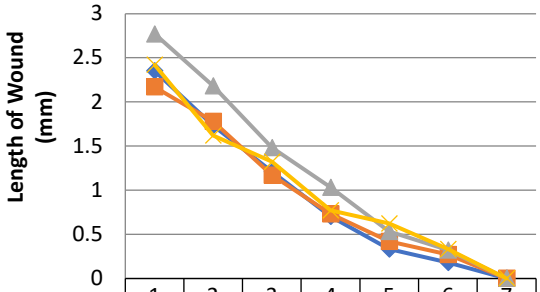
Induction of *S.aureus* and *C.albicans* were carried out using a sterile round loop. After bacterial induction, mice were left for 24 hours for infection to occur.

Measurement of wound closure at the corners of the lips was done with measurement of wound length is on the 1st-7th day after being given treatment. The length of the wound was measured on the side of the wound using a caliper.

Data will be analyzed with the *Statistical Package Social Science* (SPSS) 22.0 software application using the repeated Anova-test which begins with the Shapiro-Wilk normality test and continued with the Friedman non-parametric influence test. The value obtained is significant ($p<0.05$) then a Mann-Whitney test is performed to compare the 2 treatment groups.

RESULT

Based on the results of research that has been done on experimental animals, obtained the average value of the measurement of the wound length at the corner of the wistar strain of male white rats (Fig 1).



	1	2	3	4	5	6	7
KP 1	2.35	1.73	1.21	0.7	0.33	0.18	0
KP 2	2.17	1.78	1.17	0.73	0.42	0.27	0
KP 3	2.77	2.18	1.48	1.03	0.53	0.32	0
KP 4	2.42	1.62	1.32	0.77	0.62	0.33	0

Figure 1 The mean reduction in wound length in each treatment group

DOI 10.35856/mdj.v11i1.506

Table 1 Average values of Friedman's non-parametric test based on the mean reduction in wound length in each treatment group

KP	H-1	H- 2	H-3	H- 4	H- 5	H- 6	H-7	Friedman test
	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	Mean±sd	(p-value) **0.000***
K1	2.35±0.42	1.73±0.37	1.21±0.35	0.70±0.23	0.33±0.10	0.18±0.07	0±0	0.000*
K2	2.17±0.52	1.78±0.39	1.17±0.53	0.73±0.28	0.42±0.18	0.27±0.10	0±0	
K3	2.77±0.38	2.18±0.24	1.48±0.39	1.03±0.33	0.53±0.20	0.32±0.15	0±0	
K4	2.42±0.49	1.62±0.58	1.32±0.43	0.77±0.31	0.62±0.26	0.33±0.17	0±0	

In table 1 it can be seen that the mean reduction in wound length in each treatment group shows that all experimental animals from each treatment group experienced a change in wound length that was reduced every day. It is known that treatment group 1 is the group with the greatest reduction when compared to all treatment groups.

It shows that group 4 induced by *C.albicans* using mi-conazole and group 3 induced by *S.aureus* using gen-tamicin have a steeper curve decrease than the other groups. However, sea grape extracts in groups 1 and 2 induced by *S.aureus* and *C.albicans* still have a signi-ficant effect and the graph on day 7 shows a flat graph due to closure of the wound.

Table 1 also shows the values of Friedman's non-parametric influence tests conducted on each data group. In the table, p value was 0.000 is obtained in each group. This significance value meets the requirements of an independent variable said to have a significant effect on the dependent variable ($p < 0.05$). Therefore, it can be concluded that administration of extract sea grapes in group 1 which were induced by *S.aureus* by using sea grape extracts and group 2 which were induced by *C. albicans* by using sea grape extracts significantly affected the wound closure process at the corners of the rats lips. Similarly, group 3 induced by *S.aureus* using gentamicin and group 4 induced by *C.albicans* using miconazole significantly affected the wound closure process at the corner of the lips of white male wistar rats.

Table 2 Mann Whitney test scores for treatment group 1 and treatment group 3

KP	H-1	H-2	H-3	H-4	H-5	H-6
K1	4.75	4.75	5.08	4.50	4.42	4.58
K3	8.25	8.75	7.92	8.50	8.58	8.42
p-Value	0.078	0.021	0.154	0.046	0.034	0.051

Table 2 shows the results of different tests between treatment group 1 which was induced by *S.aureus* using sea grape extract and treatment group 3 which was induced by *S.aureus* using gentamicin, the average measurement of residual wounds in group 1 was lower, compared to group 3. The significant value obtained meets the data assumptions said to be significant which means that there is a significant difference in the remaining wound length between treatment group 1 and treatment group 3 ($p<0.05$).

Table 3 shows the average measurement in group 2 induced by *C.albicans* using sea grape extract is lower compared to group 4 induced by *C.albicans* using miconazole. It can be concluded that the difference in the remaining wound length between treatment group 2 and treatment group 4 is not significant.

Table 3 Mann Whitney test group 2 and treatment group 4 test scores

KP	H-1	H-2	H-3	H-4	H-5	H- 6
K2	5.67	7.17	5.92	6.25	5.33	6.17
K4	7.33	5.83	7.08	6.75	7.67	6.83
p-value	0.388	0.517	0.569	0.805	0.253	0.740

Table 4 Mann Whitney test group 1 and treatment group 2 test scores

KP	H-1	H-2	H-3	H-4	H-5	H-6
K1	7.25	6.83	6.75	6.25	5.33	5.00
K2	5.75	6.17	6.25	6.75	7.67	8.00
p-value	0.440	0.742	0.803	0.804	0.245	0.132

Table 4 shows the average measurement in treatment group 1 induced by *S.aureus* by using sea grape extract was higher, namely 7.25, 6.83, and 6.75. compared to group 2 induced by *C.albicans* by using sea grape extracts ie 5.75, 6.17, and 6.25. From these results. It can be concluded that the difference in wound length between treatment group 1 and treatment group 2 is not significant, which means the effect of the sea grape extract treatment group as an antibacterial material is different but not significant with the effect of the sea grape extract treatment group as antifungal to wound closure in the corner of the lip of the white male wistar strain.

DISCUSSION

In this study, the results of extracts from sea grapes affect the closure of the wound at the corner of the lips of male wistar white rats induced by *S.aureus* and *C. albicans*.

Statistical test showed that sea grape extract had a significant effect on wound closure at the corner of the lips of white male rats both induced with the *S.aureus* and those induced with *C.albicans*. This is because sea grape extract has antibacterial and antifungal properties. Based on the research of Marfuah et al, on the study of the potential of sea grape extract as an antibacterial against the bacteria *E. Coli* and *S.aureus* explained that qualitatively extracts from *Caulerpa sp* contain tannin, flavonoid,

and alkaloid compounds. Tannin compounds are bioactive compounds that have the highest content compared to other compounds. The ability of tannins as an antibacterial can be seen from the action that is able to pass through the cell membrane because it can precipitate on proteins.[3]

Antifungal activity of sea grape extract is caused by the presence of flavonoids and saponins. This is in accordance with the research of Ariani et al on the use of acacia leaf saponins (*Acacia auriculiformis A. Cunn*) as natural foaming and antibacterial agents in liquid soap states that the antifungal activity produced by grapes sea is thought to be caused by secondary metabolite compounds contained in ethanol and ethyl acetate extracts from sea grapes, namely flavonoid and saponin compounds. The mechanism of inhibition of saponins occurs through disruption of cell membrane permeability thereby causing cell lysis.[9]

Based on the results of the research shown in table 1 shows that the treatment group 1 which was induced with *S.aureus* and treated with sea grape extract showed a greater change in wound length compared to group 2 induced by *C.albicans*. *S.aureus* is a gram-positive bacterium that has a wall structure cells with more peptidoglycan, less lipids and cell walls contain polysaccharides or theatricic acid. The constituent components of peptidoglycan include amino acids and sugars. Teikoat acid is a water-soluble polymer, which functions as a transport of positive ions to get in or out. It is the water-soluble nature that indicates that the cell wall of gram-positive bacteria is more polar.[11] Flavonoid compounds and tannins are polar parts so that it is easier to penetrate polar peptidoglycan layers than non-polar lipid layers. This causes *S.aureus* to be sensitive to sea grape extract containing saponins and flavonoids.[5] The *C.albicans* are organisms that have two forms and shapes simultaneously or dimorphic organisms. *C.albicans* wall is dynamic with a layered structure, consisting of several different types of carbohydrates.[12]

Table 2 shows the different test results between treatment group 1 which was induced by *S.aureus* using sea grape extract and treatment group 3 which was induced by *S.aureus* by using gentamicin. On day 1 to day 4, there was a significant difference in the remaining wound length between treatment group 1 and treatment group 3. This difference can be caused by several factors, such as the polarity of the solvent used which will affect the diffusion process of the extract into the medium, type of bacteria, bacterial count, inoculum size, bacterial metabolic activity, and environmental conditions including temperature, pH, incubation time and components of the medium.[13]

Nursandi's research about the chemical characteristics of local seaweed and its potential as a source of antioxidants states that sea grape extract contains alkaloid, phenolic, flavonoid and triterpenoid compounds, with different levels. Plant natural antioxidant compounds are generally phenolic or polyphenolic compounds which can be in the form of flavonoids, cinnamic acid derivatives, coumarin, tocopherols and polyfunctional organic acids. Flavonoids which have antioxidant activity include flavones, flavonoids, isoflavones, catechins, and chalcons. While cinnamic acid derivatives include caffeic acid, ferulic acid, chlorogenic acid.[10]

In table 3, treatment group 2 which was induced by *C.albicans* using sea grape extract and treatment group 4 which was induced by *C.albicans* using miconazole showed that in wound healing at the corner of the lip of white male wistar there was no significant difference marked by p-value more than 0.05. This shows that the sea grape extract was significant in the closure of the wound at the corner of the lips of male white wistars strain induced by *C.albicans* because it had the same effect as its positive control, namely miconazole.

Based on the research results of Maurya et al about the chemical characteristics and antimicrobial activity of seaweed extract explained that sea grapes are considered as a source of bioactive compounds because they are able to produce secondary metabolites with various varieties. Antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green sea algae.[14]

Based on the results of previous studies conducted by Ikbal about the toxicity test of methanol extract of green seaweed on tiger shrimp larvae (*Penaeus monodon*) showed that methanol extract of macroalgae has an active ingredient that is effective as an antibacterial against the pathogenic bacteria *Vibrio harveyi*.[15] In addition, another study conducted by Kolanjinathan, et al, on the pharmacological importance of seaweed: a review of the world journal of fisheries and marine science shows that the stolon part of *Caulerpa sp.* has the highest antibacterial activity compared to the apical and basal parts.[16]

Overall sea grape extract showed significant data on wound closure at the corners of the lips of male wistar strains induced by *S.aureus* and *C.albicans*. So that this sea grape extract can be an alternative medicine for healing wounds on the corners of the lips in patients with angular cheilitis which one of the causes is *S.aureus* and *C.albicans* infections.

From these results, it can be concluded that a) sea grape extract has an effect on wound closure at the corners of the lips of male white wistar rats induced by *S. aureus* and *C.albicans*; b) sea grape extract has the same wound healing effect as gentamicin as a positive control in the wound closure process at the corners of the wistar rats male lips induced by *S.aureus* and micona-

DOI 10.35856/mdj.v11i1.506

zole as positive control in the wound closure process at the corners of the lips of male white rats induced by *C. albicans*; and c) sea grape extract has better antibacterial effectiveness compared to antifungals in the closure of the angular wounds of white wistar rats that are induced by *S.aureus* and *C.albicans* although there is no significant difference.

So, it is important to observe histopathologically using microscopes to see cellular conditions in the injured tissue starting from inflammation until the wound heals and regarding the dose of the effect of sea grape extract on wound closure at the corner of the lip.

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DOI 10.35856/mdj.v11i1.506