

Potential Extract Of Red Algae (*Gracilaria verrucosa*) from Aceh's Coast Against *Streptococcus mutans*

Potensi Ekstrak Alga Merah (*Gracilaria verrucosa*) dari Pesisir Pantai Aceh Terhadap *Streptococcus mutans*

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ABSTRACT

Streptococcus mutans is known as the main agent of dental caries. Characteristics of *Streptococcus mutans* are a Gram-positive, facultatively anaerobic bacteria with an acidophilic as an advantage characteristic of this bacteria than other cariogenic bacteria. This characteristic gives the ability to survive and thrive in a very low pH atmosphere. *Gracilaria verrucosa*, the red algae, is one of many marine products of Aceh's coast, which contains antibacterial compounds such as flavonoids, tannins, and phenols. This research aims to explore the antibacterial potency of red algae extract of *Gracilaria verrucosa* from Aceh's coast towards *Streptococcus mutans* by observing *Minimum Inhibitory Concentration* (MIC) and *Minimum Bactericidal Concentration* (MBC). The maceration method was used to produce the extraction of *Gracilaria verrucosa* with 96% ethanol as solvent. The extract concentration used for this study was 20%, 40%, 60%, 80%, and 100%. The results showed that the growth of *S. mutans* colonies had started to decrease at a concentration of 20%, i.e. 995.3×10^4 CFU/ml, and the least colony growth occurred at a concentration of 100%, i.e. 54×10^4 CFU/ml. To conclude, *Gracilaria verrucosa* extract has antibacterial potential against *S. mutans* with MIC at a concentration of 20%, whereas MBC is not found.

Keywords: Antibacterial, Caries, *Streptococcus mutans*, *Gracilaria verrucosa*

ABSTRAK

Streptococcus mutans dikenal sebagai agen bakteri kariogenik utama penyebab terjadinya karies gigi. Bakteri tersebut memiliki karakteristik berupa bakteri Gram positif fakultatif anaerob yang memiliki sifat asidofilik sehingga menjadikannya lebih dominan ditemukan pada penderita karies dibandingkan bakteri kariogenik lainnya. Karakteristik ini memberikan kemampuan untuk bertahan hidup dan berkembang dalam suasana pH yang sangat asam. Ganggang merah (*Gracilaria verrucosa*) merupakan salah satu hasil pesisir pantai Aceh yang mengandung senyawa antibakteri seperti flavonoid, tanin dan fenol. Penelitian ini bertujuan untuk mengetahui potensi antibakteri ekstrak tanaman laut tersebut terhadap *Streptococcus mutans* dengan mengamati Konsentrasi Hambat Minimum (KHM) dan Konsentrasi Bakterisida Minimum (KBM). Metode maserasi digunakan untuk menghasilkan ekstrak *Gracilaria verrucosa* dengan pelarut etanol 96%. Konsentrasi ekstrak yang digunakan dalam penelitian ini adalah 20%, 40%, 60%, 80% dan 100%. Hasil penelitian menunjukkan bahwa pertumbuhan koloni *S. mutans* sudah mulai menurun pada konsentrasi 20% yaitu $995,3 \times 10^4$ CFU/ml dan pertumbuhan koloni paling sedikit terjadi pada konsentrasi 100% yaitu 54×10^4 CFU/ml. Disimpulkan bahwa ekstrak *Gracilaria verrucosa* memiliki potensi antibakteri terhadap *S. mutans* dengan KHM pada konsentrasi 20% namun KBM tidak ditemukan.

Kata kunci: Antibakterial, karies, *Streptococcus mutans*, *Gracilaria verrucosa*

INTRODUCTION

The province of Aceh which is located at the far west of Indonesia has a territorial area surrounded by the ocean. Seaweed (*Gracilaria verrucosa*), one of the ocean's natural resources that is advantageous to people, is one such resource. Red algae belonging to the genus *Gracilaria verrucosa* are a form of seaweed known as agarophytes because they make agar.¹ Algae contain many important metabolite components that can be utilized by the body. The components of seaweed consist of two groups: primary and secondary. The primary metabolite components are vitamins, minerals, fibers, alginates, and agar. The secondary metabolite component of seaweed has the potential as a producer of various bioactive metabolites with very wide activation as antibacterial, antiviral, anti-fungal, and cytotoxic.^{2,3}

In medicine, recently seaweed was developed as a suture raw material, mixtures in medicines, and so on.⁴ Meanwhile in dentistry, based on research of Nick Jakubvics (a reference is needed here), the components of seaweed could control bacteria such as *Streptococcus mutans* (*S. mutans*) as the main pathogen of dental caries.⁵

Because it lives on the dental surface, this bacterium is frequently identified in the human oral cavity after the tooth has emerged. Initially, *S. mutans* are known to be part of the normal flora in the oral cavity which plays a role in the process of carbohydrates fermentation producing an acid that eventually leads to enamel demineralization.⁶⁻⁹

The utilization of herbal plants for caries therapy is continuously developed. Previous research, about the antibacterial extract of *Gracilaria sp.* on the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella sp* showed that *Gracilaria sp.* could inhibit the growth of these bacteria.¹⁰ Studies into the effectiveness of *Gracilaria verrucosa* extract as an immunostimulant for preventing Pacific white shrimp white spot disease *Vannamei Litopenaeus* The results showed the potential benefits of implementing *Gracilaria verrucosa* extract as an immunostimulant.¹¹

There has been no research on the antibacterial potential of red algae species *Gracilaria verrucosa* against *S. mutans*.

This study was conducted to test the antibacterial potency of red algae *Gracilaria verrucosa* extract and to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) extract against *S. mutans* as the main agent of dental caries.

METHODS

In this study, we used *Streptococcus mutans* ATCC 31987. *Gracilaria verrucosa* samples obtained from Pulo Aceh one of the small islands in the province of Aceh producing seaweed.

The maceration method was used to extract *Gracilaria verrucosa*, as shown below; First, wash the sample and dry it out by not exposing it to direct sunlight for three days. The sample was then cut into 1 cm pieces with scissors and placed in a jar. Then, add ethanol (96%) and macerate for three times 24 hours at room temperature. The solution was filtered every 24 hours to remove the dregs or residue. Finally, all of the filtrate was concentrated using a rotary evaporator set to 40°C.¹⁰ The extraction results were tested for phytochemicals to determine the active compounds contained therein.

S. mutans were grown in Nutrient Agar (NA) media using the T scratch technique (streak T). The bacterium was inoculated onto a petri disc, which was then placed in an anaerobic jar and incubated at 37°C for 3 x 24 hours.¹³ Gram staining was done to ensure that *S. mutans* was not contaminated. *S. mutans* that has been cultured in NA medium were taken with the ossea needle and put into a test tube containing 5 ml of 0.9% NaCl solution. The test tube was homogenized with a vortex and equalized the turbidity with 0,5 (1.5 x 10⁸ CFU/ml) Mc. Farland solution. The next step was making serial dilution of *S. mutans*.^{13,14}

Six test tubes were produced and labeled to assess the antibacterial properties of *Gracilaria verrucosa* extract on *S. mutans*. The following conditions were used to fill each tube with 3.5 ml: Group 1 was a control group that received 0.5 ml of *S. mutans* suspension and 3.5 ml of distilled water. The positive control group received 0.5 ml of *S. mutans* suspension and 3.5 ml of Chlorhexidine 2%. Following that 3.5 ml of *Gracilaria*

verrucosa extract concentration of 20% and 0.5 ml of *S. mutans* suspension were added to the treatment group (P1). P2 was given 3.5 ml of *Gracilaria verrucosa* extract concentration of 40% and 0.5 ml of *S. mutans* solution. P3 was given 3.5 ml of *Gracilaria verrucosa* extract concentration of 60% and 0.5 ml of *S. mutans* solution. P4 was given 3.5 ml of *Gracilaria verrucosa* extract concentration of 80% and 0.5 ml of *S. mutans* suspension. The final treatment group 5 (P5) received 3.5 ml of 100% *Gracilaria verrucosa* extract and 0.5 ml of *S. mutans* solution.^{12,15}

All the tubes were shaken so the solution became homogeneous by using a vortex. Then, followed by taking 0.5 ml of suspension by using *Eppendorf* pipette from each tube and culturing by *spread plate* method on Muller Hinton Agar (MHA) medium. Suspension of all groups was flattened with L stem and incubated at anaerobic atmosphere at 37° for 3x24 hours in the incubator. Gas packs were used to make anaerobic conditions. The final step of the method was the observation of *Streptococcus mutans*. It was done by counting the number of *S. mutans* colonies that grew by using the *Colony counter*. The Minimum Inhibitory Concentration of *Gracilaria verrucosa* extract was the lowest concentration that can inhibit *S. mutans* growth which was seen from the least colony growth rate compared to the negative control while the Minimum Bactericidal Concentration of *Gracilaria verrucosa* extract was the lowest concentration which was seen from the absent of *S. mutans* growth at MHA medium compared to Chlorhexidine 2% as positive controls.¹⁴ The tests were done on MHA medium and each treatment was repeated three times.

RESULTS

The results of *S. mutans* culture on *Trypticase Soy with Sucrose and Bacitracin* (TYS20B) as selective media and Gram staining are shown in Figures 1 and 2.

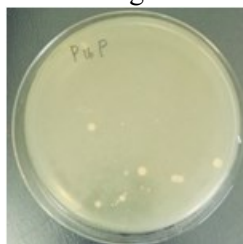


Figure 1. Colonies of *S. mutans* on TY20B media

The colonies that grew on selective media, notably TY20B, resembled *S. mutans* colonies in the form of round-shaped colonies with flat edges, yellowish-white hue, and an overwhelming yeast-like odor, as shown in the figure above. Meanwhile, the Gram staining technique was used to identify *S. mutans*, and the colonies were purple in color, coccus-shaped (round), and organized in a chain, as shown in Figure 2.

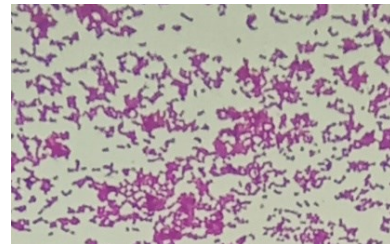


Figure 2. Gram Staining of *S. mutans*

The result of this test showed the growth of *S. mutans* colonies after being divided by their dilution rate (10^{-4}) then obtained the highest average number of colony growth in the treatment group with the concentration of 20% was $9,95 \times 10^6$ CFU/ml and the least colony growth was in the concentration of 100% that was $5,4 \times 10^5$ CFU/ml, whereas in the positive control group (CHX 2%) there was no growth of bacterial colonies and in the negative control group (distilled water) there was bacterial colonies growth that was $2,95 \times 10^7$ CFU/ml.

Table 1. the number of *S. mutans* colonies after being tested by *Gracilaria verrucosa* extract.

The concentration of tested material	Number of Colony (CFU/ml)			The average number of colonies (CFU/ml)
	P1	P2	P3	
20%	1006 $\times 10^4$	982 $\times 10^4$	998 $\times 10^4$	995,3 x 10^4
40%	782 $\times 10^4$	776 $\times 10^4$	770 $\times 10^4$	776 x 10^4
60%	388 $\times 10^4$	380 $\times 10^4$	376 $\times 10^4$	381,3 x 10^4
80%	205 $\times 10^4$	200 $\times 10^4$	198 $\times 10^4$	201 x 10^4
100%	56 x 10^4	52 $\times 10^4$	54 $\times 10^4$	54 x 10^4
Akuades	2984 $\times 10^4$	2878 $\times 10^4$	2980 $\times 10^4$	2947,3 x 10^4
Chlorhexidine 2 %	0	0	0	0

DISCUSSION

The *Gracilaria verrucosa* extraction results of phytochemical tests showed that the red algae extract of *Gracilaria verrucosa* contained steroid, terpenoid, tannin, and phenolic compounds. It also showed that the red algae *Gracilaria verrucosa* extract did not contain alkaloids, saponins, or flavonoid compounds. This might be due to several natural factors such as environmental conditions including light, water, temperature or salinity, life phase, age, geographic location, and season.^{17,18} The temperature factors could affect the photosynthesis process which then influenced the compound level in plants.^{17,8} The season factor influenced was when high rainfall which could cause the plants to get less sunlight intake. The other factors, such as salinity, were natural factors that play a role in encouraging the process of changing organic in water into minerals that could be absorbed by plants.¹⁸⁻²⁰ This might be a consideration in antibacterial activity not only associated with a single compound but also could be attributed to several compounds and metabolites combination.¹⁹

The dilution method was used in this research because this method could be used to measure Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The results of the antibacterial potency test of *Gracilaria verrucosa* extract against *S. mutans* showed that *Gracilaria verrucosa* extract was able to inhibit the growth of *S. mutans*. This was proved by decreasing number of *S. mutans* colonies grown in each treatment concentration when compared to the negative controls. The growth of *S. mutans* decreased with the high concentration of *Gracilaria verrucosa* extract. The higher the concentration, the more chemical compounds there are. The presence of tannins in *Gracilaria verrucosa* causes an antibacterial effect, which damages the cell membranes of *S. mutans*. Terpenoids in the extract also cause cell wall damage by reacting with porins, which are transmembrane proteins on the outer membrane of the bacterial cell wall, generating strong polymeric linkages that cause porin damage. This is aggravated by the presence of steroid-induced liposome leakage. The activity of these active chemicals damages the cell wall of *S. mutans*, preventing the bacteria from carrying out its duties. The

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In this study, the minimum inhibitory concentration (MIC) was 20%, whereas the minimum bactericidal concentration (MBC) was not reached since *S. mutans* grew even at the highest concentration of 100%. This could be because the *Gracilaria verrucosa* extract lacks flavonoids. The study by Almeida (2011) stated that the hydroxyl groups present in the flavonoid compound structure could cause changes in organic components and transport of nutrients which eventually resulted in toxic effects on bacteria.¹⁸

From the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), the extract of *Gracilaria verrucosa* against *S. mutans* as the main agent of dental caries can be concluded that the extraction of red algae *Gracilaria verrucosa* had antibacterial potency which could inhibit the growth of *S. mutans*. The Minimum Inhibitory Concentration (MIC) of red algae extract of *Gracilaria verrucosa* against *S. mutans* was 20% without Minimum Bactericidal Concentration (MBC).

CONCLUSION

Based on the result of this research concluded that *Gracilaria verrucosa* extract has antibacterial potential against *S. mutans* with MIC at a concentration of 20% whereas MBC is not found.

CONFLICT OF INTEREST

no conflict of interest.

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