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Antimicrobial Activity and Sulfated Polysaccharides Antibiofilms in Marine Algae Against Dental Plaque Bacteria: A Literature Review

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ABSTRACT

Introduction: Biofilm in dental plaque is known to become the cause of the occurrence of dental infection in the oral cavity, one of which is caries. A biofilm is a community of certain microorganisms attached to the surface, which is generally encapsulated and protected by an extracellular matrix consisting of various biopolymers. Therefore, the elimination of microbial biofilms is not easy, and its existence shows resistance to antimicrobial. Therefore, it needs an agent of antipyretic in the form of antimicrobial and antibiofilm that can prevent the presence of biofilm formation in dental plaque.

Objective: To see the activities of antimicrobial and antibiofilm of sulfated polysaccharides marine algae against dental plaque bacteria.

Method: Scientific evidence and clinical cases were taken from literature to support this review and information about the relationship of teeth to the incidence of stunting in children was collected.

Results/Discussion: Marine algae have bioactive compounds with their

potential to act as antimicrobial agents and antibiofilm for oral pathogens that cause oral infections in the human body.

Conclusion: Fucoidans content in several types of marine algae are classified as Sulfated Polysaccharides that are effectively working as an antimicrobial and antibiofilm

Keywords: Marine algae, Biofilms, Dental plaque, Bacterial pathogens, Oral infections, Sulfated Polysaccharide, Antimicrobial, Antibiofilms

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INTRODUCTION

Dental caries is one of the diseases that cannot be transmitted mostly found around the world with its prevalence of 35% for all ages.¹ This disease is one of the diseases generally experienced by children and becomes the major cause of early teeth loss in children.^{1,2} According to statistical data analysis by the World Health Organization (WHO), the prevalence of dental caries in children is around 60-80% in which in the end will cause pain as well as local infection and systemic.³⁻⁴ Therefore, it can influence nutrition, growth and development, general health, as well as children's life quality.⁴

Biofilms in dental plaque are known to become the cause of dental infection in the oral cavity one of which is caries. The presence of oral pathogens in the dental surface in dental plaque is proven to cause caries that contribute to decays, missing, and filling in teeth.⁵ Dental caries is often associated with an increase in acidogenic Streptococcus mutants and lactobacilli, which can metabolize sugars into acids and consequently cause demineralization of enamel.^{5,6} Due to a direct bacterial involvement is very important in the pathogenesis of infection, therefore, ideal to investigate the possibility of controlling bacterial activity to prevent infection.⁵

Marine algae have attracted much attention as a new source for the bioactive compound because they possess potential anti-therapeutic effects against various diseases. In the case of antimicrobial agents, Marine algae have been tested globally because of the sulfated polysaccharides content which consists of fucoidan, ulvan, and carrageenan which are potential as antimicrobial agents against various human

pathogenic bacteria.^{5,7} A biofilm is a certain community of microorganisms attached to the surface, which generally encapsulated and protected by an extracellular matrix consisting of various biopolymers. Therefore, the elimination of microbial biofilms is not easy, and their presence shows antimicrobial resistance. Inhibiting the formation of early biofilms from dental plaque bacteria can be a strategy to prevent dental caries.⁵

MATERIALS AND METHOD

Scientific evidence and clinical cases are taken from the literature to support this review and information about the antimicrobial activity and Sulfated Polysaccharide antibiotics in Marine algae against dental plaque bacteria. A systematic literature review was carried out looking for all articles published on antimicrobial activity and marine algae polysaccharide antibiotic films against dental plaque bacteria. On May 2nd, 2020, a literature search was carried out using the following keywords: "Marine algae, Biofilms, Dental plaque, Bacterial pathogens, Oral infections, Sulfated polysaccharide, Antimicrobials, Antibiofilms." The following databases were searched: PubMed and GoogleScholar.

DISCUSSION

Marine Algae

Marine Algae is a macroscopic plant attached or free-floating in the ocean. This plant is a primitive plant without roots, stems, and leaves that are native to the Thallophyta division; Kingdom of plants. Marine Algae is classified into four groups namely Chlorophyceae (green marine algae),

Phaeophyceae (brown marine algae), Rhodophyceae (red marine algae) and Cyanophyceae (blue-green marine algae) based on the type of pigment, morphology, anatomy and reproductive structure.⁸

The marine environment is reported to have been described as an extraordinary reserve of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products.⁸ Among marine flora and fauna, Marine algae are rich in diverse sources of bioactive compounds with various biological activities.^{8,9} Marine algae have been reported to contain many substances such as alginate, carrageenan, and agar as phycocolloids which have been used for decades in the fields of medicine and pharmacy.⁸ In addition marine algae have been tested globally because of its sulfated polysaccharides content which consists of fucoïdan, ulvan, and carrageenan that are potential as antimicrobial agents against various human pathogenic bacteria.⁶

Marine Algae Bioactive Compounds

Marine algae or macroalgae providing a variety of natural metabolites and bioactive compounds with antimicrobial activity, such as polysaccharides, polyunsaturated fatty acids, phlorotannins, and other phenolic compounds, and carotenoids.⁵

1. Polysaccharides and Oligosaccharide Derivatives

The main components of green, brown, and red marine algae are usually polysaccharides that have a variety of functions and structural. The marine algae cell wall consists of various polysaccharides including alginic acid and alginates, carrageenans and agar, laminarans, fucoidans, ulvans, and their derivatives.¹⁰

The activities of their antimicrobial depend on several factors, such as distribution, molecule weight, loads density, sulfate content (in sulfated polysaccharides), and structure and confirmation aspects. In addition, oligosaccharides obtained by depolymerization of marine algae polysaccharides also induce protection against viral, fungal, and bacterial infections, in plants. Marine algae macromolecules include sulfated polysaccharides such as carrageenan and agar from red marine algae; alginate, fucan, and laminarin from brown marine algae; and cellulose and ulvan from green marine algae.¹⁰

a) Alginate

Alginates are distinguished from other hydrocolloids (agar and carrageenan) because they are isolated from brown marine algae (Phaeophyceae) specifically from the outer layers of brown algae cell walls; as the inner layer consists mostly of cellulose. This alginate molecule provides flexibility and strength to plants.¹¹

b) Carrageenan

Carrageenan can be extracted from several species of red marine algae including Kappaphycus, Gigartina, Eucheuma, Chondrus, and Hypnea, forming as much as 50% of the dry weight. Kappaphycus alvarezii, Eucheuma denticulatum, and Betaphycus gelatine are the most important red marine algae for commercial carrageenan production. The original source of Carrageenan comes from the wild red algae

Chondrus crispus, which continues to be used today but in limited quantities.¹¹

c) Agar

Agar is a mixture of at least two polysaccharides, i.e. agarose and agaropectin also extracted from red marine algae with the same structural and functional properties as carrageenan. Agarose is the dominant fraction of agar and consists of high molecular weight polysaccharides consisting of repeating units of (1N3) - β -D-galactopyranosyl- (1N4) - 3,6-anhydro- α -L-galactopyranose.¹⁰

d) Galactans

Sulfated galactans are the main extracellular polysaccharides of red marine algae (but also found in brown and green algae). A typical structure is the galactose linear chain.¹⁰

e) Laminarans

Laminarans are the main polysaccharides storage of brown marine algae (for example: Laminaria or Saccharina spp.).¹⁰

f) Fucoidans/Fucans

Fucoidans and laminarin are considered as a major water-soluble polysaccharide from brown marine algae. Fucoidans are a complex and heterogeneous group of polysaccharides and are sulfated polysaccharides consisting of L-fucose and sulfate esters with a small number of different molecules, which can vary from monosaccharides (ie, mannose, arabinose, glucose, galactose, xylose, etc.), acid monosaccharides, acetyl groups become proteins.¹⁰

g) Ulvan

Ulvan is a water-soluble sulfated polysaccharide extracted from the intercellular space and on the walls of green marine algae fibers (especially Ulva sp.) And accounts for 18% to 29% of the dry weight of marine algae. This polysaccharide consists mainly of units of glucuronic acid and iduronic acid together with rhamnose and xylose sulfate, connected by α and β -1 -4 bonds.¹⁰

1) Lipids, Fatty Acids and Sterols

Lipid content in marine algae ranges from 0.12% to 6.73% (dry weight) and consists mostly of phospholipids, glycolipids, and non-polar glycerolipids (neutral lipids).¹⁰

2) Phenolic Compounds

Phenolic compounds are a secondary metabolite because it is involved directly in the primary process such as photosynthesis, cell division, or algae reproduction. They are characterized by an aromatic ring with one or more hydroxyl group and antimicrobial action due to the changes in the permeability of microbe cells and the loss of internal macromolecule or by the interference in the function of the membrane and the loss of cell integrity and finally the death of the cell. The presence of simple phenol, such as hydroxycinnamic and benzoic acids and their derivatives, and flavonoids are reported in green marine algae, but brown marine algae contain higher phenolic compounds than green and red marine algae. The typical profile of

phlorotannin from brown marine algae with antimicrobial activity consists mainly of phloroglucinol, eckol, and dieckol.¹⁰

3) Pigmentation

Marine algae as photosynthetic organisms can synthesize three basic classes of pigments found in marine algae: carotenoids and phycobiliprotein, allowing the classification of marine algae to become Chlorophyceae (green algae), Phaeophyceae (brown algae) and Rhodophyceae (red algae). The green color is caused by the presence of chlorophyll a and b, the greenish-brown color is associated with fucoxanthin, chlorophylls a and c, and for the red color they are phycobilins, such as phycoerythrin and phycocyanin.¹⁰

Antibacterial Compounds in Marine Algae and its Functions

Marine algae are considered as the source of the bioactive compound because they are able to generate various secondary metabolites marked by a wide spectrum of biological activities with antiviral activities, antibacterial, and antifungal that acts as a potential bioactive compound that is interesting for the application of pharmacy.^{8,10} However, screening method has identified an antibacterial compound in secondary metabolites class of Marine algae such as Phaeophyceae (brown), Rhodophyceae (red), Chlorophyceae (green), Chrysophyceae (gold) and Bacillariophyceae (diatom).¹² Rodriguez et al, Bhacuni and Rawat, Priyadarshini et al, in Kausalya, et al⁹ reported that marine algae are a very good source. Components like polysaccharides, tannins, flavonoids, phenolic acids, bromophenol, and carotenoids have shown different biological activities. Depending on their solubility and polarity, different solvents show different antimicrobial activity. Thus, chemical compounds must be extracted from different marine algae to optimize their antibacterial activity by choosing the best solvent system.⁹

The potential of marine algae as a source of active compounds against pathogenic microorganisms has been confirmed in various studies. Padmakumar and Ayyakkannu in Perez, et al¹³ filtered 80 species against pathogenic bacteria and fungi. From the algae, 70% showed antibacterial activity but only 27.5% showed antifungal activity. Among the species tested, *S. aureus*, *Vibrio* spp and *Trichophyton mentagrophytes* were the most vulnerable, while *P. aeruginosa* and *Aspergillus flavus* were the most resistant.¹² Al Hazzani, et al in Perez, et al¹² reported the activities of antimicrobial in vitro was higher from the extract of methanol and acetone from the brown marine algae (*L. japonica*, *U. pinnatifida*, *E. bicyclis*) compared to the red marine algae *P. tenera* against Gram-positive and Gram-negative bacteria, some antibiotic resistant such as *S. aureus* and *P. aeruginosa* were resistant to methicillin, and resistant to yeast, *C. Albicans*.¹³

Patra, et al in Perez, et al¹³ reported that *Enteromorpha linza* L essential oil, contains high amounts of acid (54.6%) and alkenes (21.1%), was effective against *E. coli* and *S. typhimurium*. Essential oils induce bactericidal effects

through structural membrane damage caused by deposition in the cytosol or through the degradation of bacterial intracellular enzymes that result in cellular lysis.¹³ Spavieri, et al in Perez et al.¹² tested the antimicrobial activity of coarse extracts from 21 species of brown marine algae from English and Irish waters but only *Bifurcaria bifurcata* extracts did not have the ability to melt the *Mycobacterium tuberculosis* bacteria.¹³

Biofilm in Dental Plaque

Biofilm can be defined as a community of certain microorganisms attached to the surface, which is generally encapsulated and protected by an extracellular matrix consisting of various biopolymers.⁶ Biofilms are largely formed by interactions between microbial aggregates, strains of filamentous bacteria, organic and inorganic particles which are tightly joined by EPS. Biofilms mainly consist of 96-97% water and additional polymeric substances (3-4%) (EPS layer) which include polysaccharides, proteins, and nucleic acids (DNA and RNA).⁷

Oral biofilms are attached to the solid surface such as enamel, cement, or dental implant, and planted in the polysaccharide matrix. Oral biofilms help bacteria to attach in the surface and responsible for antibiotic resistance.¹⁴ Among bacteria existed in the formation of biofilm, gram-negative bacteria such as *Pseudomonas aeruginosa*, *Fluorescens Pseudomonas*, *Escherichia coli*, *Vibrio cholera* are currently studied for the formation of biofilm, meanwhile *Staphylococcus aureus* and *Staphylococcus epidermidis* are of Gram-positive biofilm bacteria mostly studied. In addition, the species of *Streptococcus* such as *Streptococcus* Group A, *Streptococcus* groups of Viridans, *Haemophilus influenza*, and *Actinomyces israelii* are also included in the bacteria with its role in the formation of biofilms in various process of infection.¹⁵

Dental plaque is originated from the formation of pellicle obtained. The pellicle is a saliva component organizing glycoprotein, mucin, a protein-rich in proline, alpha-amylase, and other protein layers formed on the tooth surface immediately after cleaning. A number of interactions facilitate the adhesion of these bacteria such as hydrogen bonds, calcium bridges, van der Waals forces, acid-base interactions, hydrophobic interactions, and electrostatic interactions that occur between various glycoproteins, salivary components and tooth surfaces that cause conformational changes for proteins that form pellicles. Adhesion to living tissue is mediated through certain molecular components such as lectin or adhesin ligands. The results of this interaction are beneficial for the colonization of primary invaders with a reversible adhesion process assisted by the secretion of extracellular polysaccharide matrices (EPS) which help bacteria to stay bonded together and attach to the pellicle.¹⁵

Streptococcus mutans, *Streptococcus sanguis*, *Streptococcus oralis*, *Streptococcus gordonii*, and *Lactobacillus addophilus* is a major pioneer organism in plaque formation, which is competitive at low pH (due to anaerobic metabolism).^{5,15} The initial attachment of bacteria to a surface can often

cause an increase in EPS synthesis. Among the plaque bacteria, *Streptococcus mutans* and *Lactobacilli*, which are acidophilic and aciduric, respectively, have been shown to be able to demineralize tooth enamel. Gradually, the surface structure of bacteria for adhesion, such as fimbria, fibrils, and other membrane protein/glycoproteins, acts as a cue to interact with the pellicle.^{14,16}

The Role of Marine Algae as Antimicrobial and Antibiofilm

Dental caries is an oral infection mediated by biofilm, the presence of the oral pathogen in the dental surface in dental plaque has been proven to cause caries, transmitted dental infection, and general transmission.^{7,15} Cariogenic bacteria

including *S. mutans* ferment carbohydrate particles and produce organic acids that are able to dissolve dental minerals which then produce caries.^{7,17,18,19,20} Biofilms are generally encapsulated and protected by an extracellular matrix consisting of various biopolymers. Therefore, eliminating microbial biofilms is not easy, and their presence shows antimicrobial resistance.⁶ Intense research clearly shows that there is a strong positive correlation between the structure of sulfated polysaccharides and their bioactivity. Previous reports also showed the potential of sulfated polysaccharides such as fucoidan, ulvan and carragenan as antimicrobial agents against many pathogenic bacteria in humans.⁷

Synthesis Table 1: Antibacterial Compounds in Marine Algae and its Functions

No	Authors and Titles	Year	Conclusion and Results
1.	M.Kausalya G.M. Narasimha Rao Antimicrobial activity of marine algae Journal : J. Algal Biomass Utln. 2015, 6 (1): 78- 87	2015	The strongest antibacterial activity is demonstrated by methanol extract by <i>Sargassum polycystum</i> . Methanol has a higher antibacterial activity than extracts obtained with other organic solvents. <i>Sargassum polycystum</i> and <i>Sargassum tenerrimum</i> using four different solvent extracts against eighteen different pathogens show significant antimicrobial activity.
2.	María Jose Perez Elena Falque Herminia Domínguez Antimicrobial Action of Compounds from Marine Seaweed Journal: Mar. Drugs (MDPI) 2016. 14(52); 1-38	2016	Marine algae is one of the largest biomass producers in the marine environment and represents a potential source of diverse and unique new compounds. Many substances obtained from marine algae, such as alginate, carragenan, and agar have been used for decades in traditional medicine, pharmacology and food. Other compounds have bacteriostatic or antibacterial, antiviral, antitumor, anti-inflammatory, and antifouling activities. Therefore, marine algae can provide promising bioactives that can be used in the treatment of human diseases, or new antimicrobial agents to replace synthetic antibacterial agents used in agriculture and the food industry.

Synthesis Table 2: Antibacterial Compounds in Marine Algae and its Functions

No	Writer and Title	Year	Conclusion and Results
1.	Joon-Young Jun, Min-Jeong Jung , In-Hak Jeong , Koji Yamazaki , Yuji Kawai and Byoung-Mok Kim Antimicrobial and antibiofilm activities of sulfated polysaccharides from marine algae against dental plaque bacteria. Journal : Mar. Drugs (MDPI) 2018;16(301);1-23	2018	Fucoidan F85 from <i>Fucus vesiculosus</i> which is part of sulfated polysaccharides has antimicrobial activity compared to 11 other marine algae species, which shows growth inhibitory effect on all dental plaque bacteria with <i>S. mutans</i> is the most sensitive bacteria among the bacterial strains tested. In addition, it inhibits the growth of <i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> and two lactic acid bacteria, but not from Gram-negative bacteria. ⁶ In contrast, Fucoidan F95 does not inhibit the growth of any bacterial strains, although it also comes from <i>F. vesiculosus</i> . In addition to Fucoidan F85, two sulfated polysaccharides from brown marine algae; <i>Undaria pinnatifida</i> and <i>Kjellmaniella crassifolia</i> have growth inhibitory effects on <i>Salmonella typhimurium</i> at concentrations of 1000 µg mL ⁻¹ (both). Ampicillin MIC (as a standard antimicrobial agent) is in the range of 0.8 to 12.5 µg mL ⁻¹ for dental plaque bacteria.

2.	<p>22</p> <p>Faezah Sabirin, Jamil Ahsan Kazi, Irman Shahir Ibrahim, Muhammad Mu'az Abd Rashit Screening of Seaweeds Potential Against Oral Infections Journal : Journal Of Applied Sciences Research.2015: 11(15), Special, Pages: 1-6</p>	2015	<p>Marine algae Chlorophyta and Rhodophyta; <i>C. lentifera</i> and <i>K. alvarezii</i> each have antibacterial activity against the causative agent for oral infection; <i>S. aureus</i> and <i>S. mutans</i> using the disk diffusion method. Antibacterial activity indicates the possible value of marine algae therapy for oral infections.</p>
3.	<p>16</p> <p>J. Vishwakarma and S.L. Vavilala Evaluating the antibacterial and antibiofilm potential of sulphated polysaccharides extracted from green algae <i>Chlamydomonas reinhardtii</i> Journal : Journal of Applied Microbiology 127, 1004--1017</p>	2019	<p>Fucoidan F85 at concentrations above 250 µg mL⁻¹ completely suppresses both the formation of biofilms and the growth of planktonic cells of <i>S. mutans</i> and <i>S. sobrinus</i>.</p>

Research conducted by Jun et al⁶ stated that Fucoidan F85 from *Fucus vesiculosus* which is part of sulfated polysaccharides has antimicrobial activity compared to 11 other marine algae species, which shows growth inhibitory effects on all dental plaque bacteria with *S. mutans* being the most sensitive bacteria among tested bacterial strains.^{21,22,23,24} In addition, it inhibits the growth of *Listeria monocytogenes*, *Staphylococcus aureus* and two lactic acid bacteria, but not from Gram-negative bacteria.^{25,26,27,28} In contrast, Fucoidan F95 does not inhibit the growth of any bacterial strains, although it also comes from *F. vesiculosus*. In addition to Fucoidan F85, two sulfated polysaccharides from brown marine algae; *Undaria pinnatifida* and *Kjellmaniella crassifolia* have growth inhibitory effects on *Salmonella typhimurium* at concentrations of 1000 µg mL⁻¹ (both).^{29,30,31,32} Ampicillin MIC (as a standard antimicrobial agent) is in the range of 0.8 to 12.5 µg mL⁻¹ for dental plaque bacteria.^{33,34,35}

Research conducted by Lee et al in Jun et al⁶, Fucoidan exert potential antimicrobial activity against several cariogenic *Streptococcus* sp. (MICs, 250-500 µg mL⁻¹; minimum bactericidal concentration (MBC), 500-1000 µg mL⁻¹) and periodontopathogenic²⁰⁻²¹ bacteria *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Porphyromonas gingivalis* (MICs, 125 µg) – 1; MBCs, 250–1000 µg mL – 1).⁶

Yamashita et al, in Jun et al⁶ studied the effects of several polysaccharide diets derived from marine algae^{22,23,24,25} and terrestrial plants on the growth of some foodborne bacteria. In their report, carragenan (λ, γ and κ) in 2500 µg mL – 1 has a bacteriostatic effect on *S. enteritidis*, *S. typhimurium*, *Vibrio mimicus*, *Aeromonas hydrophila*, *E. coli* (enterotoxigenic), and *S. aureus*. However, there is no effect from γ- carragenan even at high doses 5,000 µg mL⁻¹ were observed in all strains of bacteria tested.⁶

Research carried out by Sabirin, et al³ showed that marine algae Chlorophyta and Rhodophyta; *C. lentifera* and *K. alvarezii* each have antibacterial activity²⁷ against the

causative agent for oral infection; *S. aureus* and *S. mutans* using the disk diffusion method. Antibacterial activity^{36,37} indicates the possible value of marine algae therapy for oral infections.⁵

In dental plaque, there are more than 600 species of bacteria and archaea, but about 50% of them are currently unable to be bred because of limited growth and nutritional conditions. Research conducted by Vishwakarma, et al⁷ showed that Fucoidan F85 at the concentration above 250 µg mL⁻¹ fully suppress both biofilm formation and *S. mutans* and *S. sobrinus* planktonic cell growth.⁷

CONCLUSION

Sulfated polysaccharides are bioactive compounds found in marine algae and have the ability as an antimicrobial and antibiofilm against pathogenic bacteria that cause oral infections. Fucoidan is one part of sulfated polysaccharides that has antimicrobial and antibiofilm activity against dental plaque. Thus, with the existence of antimicrobial and antibiofilm activities, this can be used as an alternative in the early prevention of dental caries by inhibiting the formation of biofilms in dental plaque

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