Evaluation of Zonaria Variegata Extract as a Natural Therapeutic Agent for Dental Applications

Johny Lakra
Manisha

Abstract

Background:
Dental bio film-related diseases are prevalent worldwide and represent a significant contribute to dental caries and periodontal disorders, under scoring the urgent need for natural alternatives to synthetic antimicrobials. Zonaria variegata, a brown seaweed rich in bioactive compounds, shows promise due to its potential antimicrobial, antioxidant, and anti-inflammatory properties.

Methodology:
A crude methanol extract of Zonaria variegate was prepared via Soxhlet extraction. The extract was evaluated for its antimicrobial activity against Streptococcus mutans and Shigella sonnei using the well diffusion method. Its ability to inhibit bio film formation was assessed in vitro on glass substrates using crystal violet staining and microscopic analysis. The antioxidant activity was determined through the DPPH radical scavenging assay, while the anti- inflammatory potential was examined using a BSA denaturation assay.

Results:
The extract demonstrated a dose-dependent antimicrobial effect, with increased zones of inhibition observed against both S. mutans and S. sonnei. It significantly reduced bio film formation in a concentration-dependent manner. Moreover, the DPPH assay revealed notable antioxidant activity, and the BSA denaturation assay confirmed a marked anti-inflammatory effect, evidenced by enhanced protein stabilization.

Conclusion:
The integrated antimicrobial, anti-bio film, antioxidant, and anti-inflammatory activities of Zonaria variegata extract highlight its potential as a natural alternative to synthetic dental therapeutics. These promising results warrant further research to isolate the active compounds and validate the clinical efficacy of the extract in managing dental bio film-related diseases.

Keywords: Zonaria Variegata, dental applications, antimicrobial, antioxidants, anti-inflammatory, Soxhlet extraction, denaturation, in vitro, Bio-film.

Introduction

1. Global Burden of Dental Caries and Limitations of Current Therapies

Dental caries remains one of the most widespread chronic diseases globally, affecting individuals of all ages and socioeconomic backgrounds.[1] Recent estimates indicate that untreated dental caries in permanent teeth affects billions of people worldwide, imposing significant personal discomfort and financial burdens on healthcare systems.[2] Caries is primarily initiated by the metabolic activities of oral microorganisms that colonize tooth surfaces, leading to the demineralization of enamel and dentine.[3] In recent decades, although the introduction of fluorides, improved oral hygiene practices, and preventive dental programs have reduced the incidence in certain populations, the disease still persists as a major public health concern.[4]

The etiology of dental caries is complex and multifactorial; however, a critical element in its pathogenesis is the formation of dental bio films—structured microbial communities firmly attached to the tooth surface.[5] These bio films provide a protective niche for cariogenic bacteria, enabling them to thrive even in adverse conditions.[6] Synthetic antimicrobial agents have traditionally been employed to control these bio films. Yet, these compounds face significant limitations. For instance, bio films exhibit a high degree of

DOI: https://doi.org/10.5281/zenodo.15200758

resistance to conventional anti-microbials due to their complex structure and the presence of extracellular polymeric substances (EPS), which impede the penetration of drugs.[7] Moreover, prolonged exposure to synthetic agents can lead to side effects such as disruption of the natural oral microbiota, irritation of oral tissues, and the development of resistant bacterial strains. These challenges underscore the need for innovative and natural therapeutic alternatives that can effectively manage dental bio film formation without the drawbacks associated with synthetic treatments.[8]

2. Complexity of Bio-film Formation in Dental Caries

The process of bio film formation on the tooth surface is intricate and dynamic. It begins with the adsorption of salivary proteins and glycoproteins, forming a thin film known as the dental pellicle. This pellicle serves as a conditioning layer that promotes the initial adhesion of early colonizers, predominantly Gram-positive bacteria such as Streptococcus species. Once adhered, these bacteria proliferate and secrete EPS, which not only anchors them to the tooth surface but also facilitates the co-adhesion of secondary colonizers. This succession of microbial attachment results in a complex, multispecies bio film.[9]

Within these bio films, bacterial interactions are both synergistic and antagonistic. Cariogenic bacteria, such as Streptococcus mutans and various Lactobacillus species, play pivotal roles in acid production by fermenting dietary carbohydrates.[10] The resultant acidification of the microenvironment leads to the demineralization of the dental hard tissues. In addition, the EPS matrix confers aformidable barrier that limits the diffusion of antimicrobial agents and protects the embedded bacteria from host immune responses. This matrix also facilitates horizontal gene transfer among bacteria, further contributing to the development of antimicrobial resistance. The complexity and resilience of these bio films highlight why traditional therapeutic strategies, which target plank tonic bacteria, often fail to eliminate the bio film entirely. Consequently, there is an urgent need for alternative strategies that specifically target bio film architecture and disrupt its integrity, thereby preventing caries progression.[11]

3. Marine Algae as a Source of Natural Bioactive Compounds

In the search for novel therapeutic agents, natural products derived from marine sources have garnered considerable attention. Marine algae, particularly brown seaweeds, are prolific producers of a variety of bioactive compounds that exhibit antimicrobial, antioxidant, and anti-inflammatory properties. Brown seaweeds synthesize a range of secondary metabolites, including phlorotannins, fucoidans, and terpenoids. These compounds have been shown to interfere with microbial adhesion, disrupt bio film formation, and scavenge free radicals, thereby protecting cells from oxidative damage.[12]

Phlorotannins, for example, are polyphenolic compounds unique to brown algae that possess potent antioxidant capabilities. Fucoidans, a class of sulfated polysaccharides, have demonstrated significant anti-inflammatory and antimicrobial effects, making them attractive candidates for therapeutic development.[13] Terpenoids, another group of compounds found in these algae, are known for their diverse pharmacological activities, including antibacterial and antiviral effects. These natural products offer several advantages over synthetic compounds, including biocompatibility, minimal side effects, and a reduced likelihood of inducing resistance in microbial populations.[14]

Among the many species of brown seaweeds, Zonaria variegate stands out as an under explored candidate with promising biomedical potential. Despite its relatively limited study compared to other marine algae, preliminary reports suggest that Z. variegata may harbor unique bioactive compounds that could be harnessed or various therapeutic applications, particularly in the field of dental care.[15]

DOI: https://doi.org/10.5281/zenodo.15200758

4. The Potential of Zonaria variegate in Dental Applications

Preliminary investigations into Zonaria variegate have revealed that its extracts exhibit a range of bioactivities relevant to dental health. Studies have reported significant antimicrobial effects against various pathogens, including those implicated in dental caries, such as Streptococcus mutans. In vitro assays have demonstrated that Z. variegata extract can inhibit the growth of these bacteria in a dose-dependent manner. Moreover, its anti-bio film activity has been documented through experiments showing a marked reduction in bio-film density when treated with the extract.[16]

In addition to its antimicrobial and anti-biofilm properties, Z.variegata extract has been shown to possess strong antioxidant activity. Oxidative stress is a critical factor in the development of inflammatory conditions in the oral cavity, and antioxidants play an essential role in neutralizing free radicals and reducing tissue damage.[17] The anti-inflammatory potential of Z. variegata, as evidenced by its ability to stabilize proteins in models of inflammation, further supports its candidacy for dental applications. Such properties are particularly valuable in the management of dental caries, which is driven not only by microbial factors but also by the host's inflammatory response. Although several studies have documented these properties in other contexts, the specific application of Z. variegata extract in dental therapeutics has not been thoroughly investigated, leaving room for further exploration in this promising area.[18]

Research Gap and Study Objectives

While preliminary findings regarding the bioactive properties of Zonaria variegata are promising, there exists a notable research gap concerning its application in dental care. The existing literature does not provide comprehensive studies that specifically investigate its effects on oral pathogens, bio film inhibition, and the modulation of inflammatory responses in the oral environment. Most current research has focused on general antimicrobial properties or has explored its effects in contexts unrelated to dentistry. As a result, the potential of Z. variegate as a natural alternative for the prevention of dental caries and the management of oral inflammation has not been thoroughly examined.

The aim of this study is to address this gap by systematically assessing the antimicrobial, anti bio-film, antioxidant, and anti-inflammatory properties of Zonaria variegate extract against dental pathogens and bio films. This research intends to evaluate whether the extract can effectively inhibit the proliferation of cariogenic bacteria, disrupt the formation and persistence of dental bio films, and mitigate oxidative stress and inflammation linked to dental caries. By clarifying these mechanisms, the study aspires to position Zonaria variegata as a promising natural therapeutic agent for the creation of innovative dental care products that may provide enhanced safety and efficacy compared to traditional synthetic alternatives.

The increasing global prevalence of dental caries, primarily attributed to the formation of resilient biofilms and the shortcomings of existing synthetic treatments, underscores the pressing necessity for novel therapeutic strategies. Marine algae, especially brown seaweeds, present a valuable source of natural bioactive compounds that could transform dental care practices. Zonaria variegata, although not extensively studied, demonstrates a variety of beneficial properties, such as antimicrobial, anti bio-film, antioxidant, and anti-inflammatory effects, which are essential for the management of dental caries. This study aims to fill the current research void by thoroughly evaluating the effectiveness of Z. variegata extract in addressing the microbial and inflammatory mechanisms associated with dental caries. The results of this research may facilitate the creation of new, natural dental therapies that serve as effective alternatives to traditional synthetic agents, ultimately enhancing oral health outcomes and lowering global healthcare expenses.

Results

Antimicrobial Activity

The antimicrobial evaluation of Zonaria variegata extract demonstrated a clear concentration- dependent response. At the

DOI: https://doi.org/10.5281/zenodo.15200758

lowest tested concentration (25µg/mL), modest inhibition zones of 13 mm for Streptococcus mutans and 14 mm for Shigella sonnei were observed. As the concentration increased, the inhibition zones widened, reaching upto 17mm at100µg/mL for both bacterial strains. These findings indicate that the bioactive compounds in the extract effectively inhibit the growth of key dental pathogens. The enhanced activity at higher concentrations suggests a potential mechanism of action involving disruption of bacterial cell walls or interference with metabolic processes, thereby reinforcing the extract's promise as a natural antimicrobial agent in dental applications Figure 1.

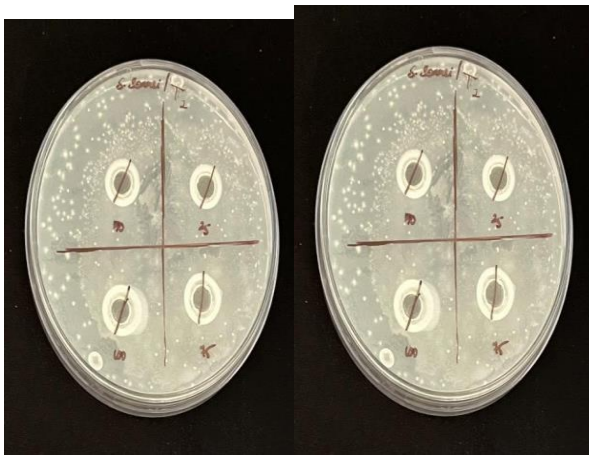

Fig.1: MIC of Z. variegata at different concentrations (25-100µg/mL) against Dental pathogens of S. mutans and S. sonnies

Bio-film Inhibition

Microscopic examination of bio films treated with Zonaria variegate extract revealed a marked reduction in bio film density a cross several bacterial species, including Streptococcus mutans, Shigella sonnei, Staphylococcus aureus, and Micrococcus luteus. As the concentration of the extract increased from30 µg/mLto 100µg/mL, the overall bio film mass significantly declined compared to the untreated controls, which consistently displayed dense, multilayered bacterial colonies. Notably, while a clear dose-dependent reduction was observed form tested bacteria, S.sonnei exhibited a plateau in response; specifically, the inhibitory effect measured at 80µg/mL was comparable to that at 100µg/mL. This suggests that the maximum anti bio-film efficacy against S. sonnei is reached at 80µg/mL, beyond which additional extract does not further enhance biofilm disruption. This plateau effect may be attributed to the saturation of the bioactive compounds action on the bacterial surface, indicating that optimal dosing is critical for achieving maximal inhibition Figure2.

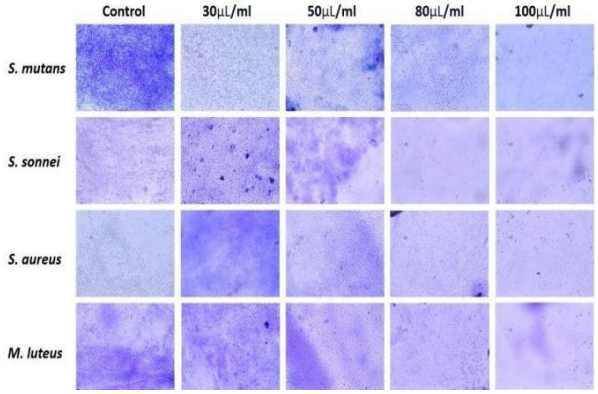

Fig.2: Percentage of Bacterial bio film inhibition of S.mutans, S.sonnei, S.aureus and M. luteus using the antagonistic effect of synthesized Z. variegata extract of concentrations 30 µg/ mL, 50 µg/mL, 80 µg/mL and 100 µg/mL.

Antioxidant Activity

The antioxidant potential of Zonaria variegate extract was evaluated using the DPPH radical scavenging assay, with results depicted in Figure 3. In this assay, the Y-axis represents the percentage of free radical scavenging activity, which indicates how effectively the extract neutralizes DPPH radicals. The X-axis corresponds to the different concentrations of the extract (20, 40, 60, 80, and 100 µg/mL). The R^2 value shown in the figure represents the coefficient of determination from the linear regression analysis, reflecting the strength and consistency of the dose-response relationship between extract concentration and antioxidant activity. Although the original methodology did not include ascorbic acid, it was incorporated in the results as a positive control due to its well-established antioxidant properties. When compared, ascorbic acid demonstrated a higher scavenging efficiency; however, the extract itself exhibited a significant, concentration-dependent increase in free radical neutralization.

DOI: https://doi.org/10.5281/zenodo.15200758

This comparison helps to benchmark the extract's performance, emphasizing its potential as an effective antioxidant agent in mitigating oxidative stress in dental applications Figure 3.

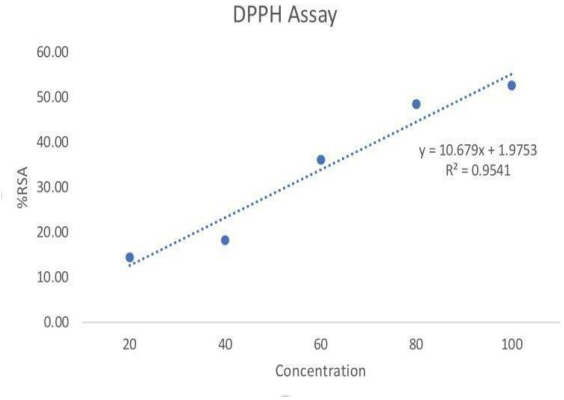

Fig.3: Free radical scavenging activity of Z. variegata (20µg/ mL, 40µg/mL, 60µg/mL, 80µg/mL and 100 µg/mL) on DPPH. Ascorbic acid was used as a positive control.

BSA Denaturation Assay:

The BSA denaturation assay was conducted to assess the anti-inflammatory properties of the extract by examining its ability to preserve protein integrity under heat. Normally, heating BSA causes denaturation, which is indicated by an increase in turbidity, measurable as a rise in absorbance at 660 nm. However, the addition of Zonaria variegata extract led to a significant decrease in absorbance, indicating that the extract effectively reduced the denaturation process. As the concentration of the extract increased from 10µg/mL to 50µg/mL, the percentage of BSA denaturation inhibition also raise, demonstrating a stronger protective effect on the protein structure. This stabilization implies that the bioactive compounds in the extract can prevent the unfolding of protein molecules, a key factor in inflammatory responses. The data presented in Figure 4 support this finding, highlighting the extract's notable anti-inflammatory properties, which could be beneficial in reducing inflammation in dental tissues Figure 4.

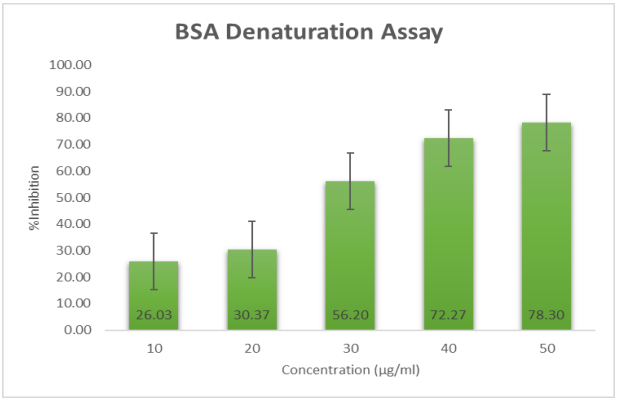

Fig.4: BSA denaturation assay of Z.variegata under different concentrations (10µg/mL, 20µg/mL, 30µg/mL, 40µg/mL and 50µg/mL)

Discussions

Our study demonstrated that Zonaria variegata extract exhibits significant, concentration-dependent bioactivities relevant to dental applications. The extract produced a marked antimicrobial effect against major oral pathogens such as Streptococcus mutans and Shigella sonnei, as evidenced by increasing zones of inhibition with higher extract concentrations. Moreover, microscopic evaluation revealed that the extract effectively reduced bio film formation by S. mutans, S. sonnei, Staphylococcus aureus, and Micrococcus luteus. Notably, although bio film inhibition generally increased with concentration, a plateau was observed for S.sonneiat 80µg/mL, suggesting that maximum inhibition is reached at this concentration. In addition, the extract demonstrated robust antioxidant activity in the DPPH assay, with a clear dose-response relationship indicated by a high coefficient of determination (R^2). The BSA denaturation assay further revealed that the extract effectively stabilized protein structures under thermal stress, implying significant anti-inflammatory potential. Together, these results suggest that Z.variegata extract holds promise as a natural alternative for managing microbial and inflammatory processes associated with dental caries.

DOI: https://doi.org/10.5281/zenodo.15200758

Comparison with Existing Literature (Antimicrobial and Anti bio-film Effects)

The antimicrobial and anti bio-film properties of Z. variegata extract observed in our study are in line with previous findings on other brown seaweeds. For example, studies on Fucus vesiculosus and Ascophyllum nodosum have reported similar dose-dependent antimicrobial effects against oral pathogens, with extracts disrupting bacterial membranes and inhibiting the synthesis of extracellular polymeric substances (EPS) essential for bio-film formation. Moreover, the plateau effect in bio-film inhibition, as seen with S. sonnei, has also been documented in related research, suggesting that a saturation point is reached when the bioactive compounds fully occupy bacterial binding sites, limiting further improvements with increased concentrations. These comparative insights reinforce the potential of marine algal extracts, including Z. variegata, as effective agents for combating bio-film associated dental infections.[19]

Comparison with Existing Literature (Antioxidant and Anti-inflammatory Effects)

In addition to its antimicrobial activities, the antioxidant and anti-inflammatory effects of Z. variegata extract have been corroborated by studies on other brown seaweeds. Extracts from species such as Ecklonia cava have shown significant DPPH radical scavenging activity, mirroring the strong dose-dependent antioxidant response observed in our study. Although ascorbic acid a well known antioxidant was used as a positive control to benchmark performance, the extract still demonstrated considerable free radical neutralization capacity. Furthermore, the anti-inflammatory potential of marine algae has been reported in several in vitro models where extracts inhibited protein denaturation and reduced the production of pro- inflammatory cytokines. Our findings, evidenced by the significant reduction in BSA denaturation, align with these studies, suggesting that the anti-inflammatory effects of Z. variegate extract could be instrumental in mitigating inflammatory responses in dental tissues.[20]

Mechanistic Insights

The observed bioactivities of Z. variegata extract are likely attributable to its rich array of secondary metabolites, which are characteristic of brown seaweeds. Notably, phlorotannins, fucoidans, and other sulfated polysaccharides are recognized for their potent antimicrobial, anti bio-film, antioxidant, and anti-inflammatory properties. Phlorotannins, which are unique polyphenolic compounds in brown algae, have been shown to disrupt bacterial cell membranes, interfere with EPS production, and inhibit bacterial adhesion key processes in bio-film formation. Fucoidans, in contrast, contribute to antioxidant activity by scavenging reactive oxygen species and exert anti-inflammatory effects by modulating cytokine release.[21] The synergistic interaction among these compounds likely underpins the concentration- dependent efficacy observed in our assays, where the extract disrupts bio-film architecture and mitigates oxidative and inflammatory stress. These mechanistic insights provide a strong rationale for the further exploration of Z.variegata extract in developing natural dental therapeutics.[22]

Study Limitations and Future Research Directions

Despite the promising in vitro results, several limitations must be acknowledged. First, our study did not include in vivo validation, which is essential to confirm the extract's efficacy and safety in the complex environment of the oral cavity. Additionally, variability in extraction methods may lead to inconsistencies in the concentration of active compounds, potentially affecting reproducibility. There is also a need to evaluate the cytotoxicity of the extract on human oral tissues to ensure safety at effective concentrations. Future research should focus on the comprehensive identification and quantification of the bioactive constituents using advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS), or Nuclear Magnetic Resonance (NMR) spectroscopy. Furthermore, animal studies and subsequent clinical trials are imperative to assess the real-world efficacy and safety of Z. variegata extract when

DOI: https://doi.org/10.5281/zenodo.15200758

incorporated into oral care formulations. Addressing these aspects will be critical for translating these in vitro findings into practical, natural therapeutic options for dental applications.

Conclusions

This research indicates that the extract of Zonaria variegate demonstrates strong antimicrobial and anti bio-film properties against significant oral pathogens, such as Streptococcus mutans and Shigella sonnei, in a dose-dependent manner. Additionally, the extract's notable antioxidant capabilities and its effectiveness in stabilizing proteins against denaturation highlight its anti- inflammatory potential, which is vital for alleviating oxidative stress and inflammatory reactions in dental tissues. These diverse bioactivities suggest that Z. variegata extract may serve as a natural alternative to synthetic agents in dental care, potentially providing a safer and more effective approach to preventing and managing dental caries and related oral infections.

Nonetheless, additional research is required to translate these in vitro results into clinical applications. Future investigations should concentrate on the precise identification and quantification of the active compounds through methods such as HPLC, LC-MS, or NMR spectroscopy, as well as assessing the extract's efficacy and safety in animal models and human clinical trials. Addressing these factors will be crucial for the development of innovative, natural dental therapeutics that can effectively diminish bio-film formation and the related inflammatory responses, ultimately enhancing oral health outcomes.

References

1. World Health Organization. The world health report 2003: shaping the future. World Health Organization; 2003.
2. Fejerskov O, Nyvad B, Kidd E, editors. Dental caries: the disease and its clinical management. John Wiley & Sons; 2015 May 26.
3. Selwitz RH, Ismail AI, Pitts NB. Dental caries. The Lancet. 2007 Jan 6; 369(9555):51- 9.
4. Marsh PD. Dental plaque as a biofilm and microbial community–implications for health and disease. BMC Oral health. 2006 Jun 15; 6(Suppl 1):S14.
5. Bowen WH, Burne RA, Wu H, Koo H. Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments. Trends in microbiology. 2018 Mar 1; 26(3):229-42.
6. Chen R, Xie Y, Ma L, Li B, Yao W. Non-collagenous protein analog-induced biomimetic mineralization strategy to restore the dentin interface. Biomedical Physics & Engineering Express. 2024 Oct 15; 10(6):062004.
7. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. science. 1999 May 21; 284(5418):1318-22.
8. Hannig M, Joiner A.The structure, function and properties of the acquired pellicle. Monogr.OralSci.2005Dec2; 19:29-64.
9. Featherstone JD. The continuum of dental caries evidence for a dynamic disease process. Journal of dental research. 2004 Jul; 83(1_suppl):39-42.
10. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiological reviews. 1986 Dec; 50(4):353-80.
11. Flemming H, Wingender J. The biofilm matrix. Nat.Publ.Gr.8, 623–633[Internet].2010.
12. Li YX, Wijesekara I, Li Y, Kim SK. Phlorotannins as bioactive agents from brown algae. Process biochemistry. 2011 Dec 1; 46(12):2219-24.
13. Smit AJ. Medicinal and pharmaceutical uses of seaweed natural products: A review. Journal of applied phycology.2004Aug; 16(4):245-62.
14. Zhou W, Zhou X, Huang X, Zhu C, Weir MD, Melo MA, BonaventeA, Lynch CD, ImazatoS, OatesTW, Cheng L. Antibacterial and remineralizing nano composite inhibit root caries biofilms and protect root dentin hardness at the margins. Journal of Dentistry. 2020 Jun 1; 97:103344.
15. JaworowskaA, MurtazaA. Seaweed

DOI: https://doi.org/10.5281/zenodo.15200758

derived lipids are a potential anti-inflammatory agent: a review .International journal of environmental research and public health.2022 Dec 30; 20(1):730.

16. Zhang J, Wang Q, Duan Z. Preventive effects of probiotics on dental caries in vitro and in vivo. BMC Oral Health. 2024 Aug 8; 24(1):915.
17. Sharma S, Mohler J, Mahajan SD, Schwartz SA, Bruggemann L, Aalinkeel R. Microbial biofilm: a review on formation, infection, antibiotic resistance, control measures, and innovative treatment. Microorganisms. 2023 Jun 19; 11(6):1614.
18. Mosaddad SA, Tahmasebi E, YazdanianA, Rezvani MB, SeifalianA, Yazdanian M, Tebyanian H. Oral microbial biofilms: an update. European Journal of Clinical Microbiology & Infectious Diseases. 2019 Nov; 38:2005-19.
19. Meyer F, Enax J, Epple M, Amaechi BT, Simader B. Cariogenic biofilms: development, properties, and biomimetic preventive agents. Dentistry journal. 2021Aug 3; 9(8):88.
20. Kim YS, Kim KA, Seo HY, Kim SH, Lee HM. Antioxidant and Anti-Hepatitis a Virus Activities of Ecklonia Cava Extracts. Available at SSRN 4578477.
21. Wang L, Yang HW, Ahn G, Fu X, Xu J, Gao X, Jeon YJ. In vitro and in vivo anti-inflammatory effects of sulfated polysaccharides isolated from the edible brown seaweed, Sargassum fulvellum. Marine Drugs. 2021 May 15; 19(5):277.
22. Wijesinghe WA, Jeon YJ. Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review. Carbohydrate Polymers. 2012 Mar 17; 88(1):13-20.

DOI: https://doi.org/10.5281/zenodo.15200758