Investigating the Potential of Zonaria Variegata Extract for Dental Applications

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Abstract
Zonaria variegata, a species of brown seaweed, has gained attention for its diverse bioactive compounds with potential antimicrobial and antioxidant properties. This study investigates the efficacy of Z. variegata extract in dental applications, particularly its antimicrobial activity against oral pathogens and its potential to inhibit bio film formation. The extract was prepared using methanol extraction and tested against Streptococcus mutans and Shigella Sonnei using the well diffusion method. Additionally, its bio film inhibition and antioxidant properties were evaluated through in vitro assays. The results demonstrated a dose-dependent antimicrobial effect, with significant bio film inhibition and antioxidant activity. These findings suggest that Z. variegata extract could serve as a natural alternative to synthetic antimicrobial agents in dental care. Further studies are required to identify specific bioactive compounds and assess their clinical efficacy.

Keywords: Zonaria Variegata, dental applications, antimicrobial, antioxidants, Bio-film.

Introduction

Dental complications by bacteria and its bio films

Over the past few decades, numerous reports from around the world have indicated a significant decline in dental caries, with this downward trend continuing across populations. Major factors contributing to this success include the use of systemic and topical fluorides, toothpaste, sealants, improved diets, oral health education, and advancements in dental care.[1] However, recent studies have raised concerns by reporting alarming increases in dental caries.[2] These increases are observed in both children and adults, affecting Primary and permanent teeth, and involving both coronal and root surfaces.[3]

Trillions of microorganisms reside in the human body, with the oral cavity being one of the main habitats. Approximately 700–1000 different types of microorganisms live in the human mouth. Oral microbiota plays a key role in the development and occurrence of diseases of the mouth, including dental caries, periodontal disease, and oral cancer.[4] Most of these species act as commences that protect the oral cavity from colonization of disease-causing organisms, but some of them are directly responsible for the occurrence of dental caries and periodontal disease.[5] Dental disease is unquestionably a public health concern and one of the most common illnesses worldwide, especially dental caries, a condition linked to biofilms.[6]

The oral microbiota on the tooth surface forms polymicrobial communities known as dental biofilms, which create a pathological environment for cariogenic microorganisms. Dental caries is now recognized as a bio film-induced disease rather than an infectious one, originating in the bio film that covers the tooth surface. The formation of dental bio film starts when a salivary glycoprotein film (dental pellicle) coats a tooth, followed by colonization by Gram- positive bacteria like Streptococci, which produce extra cellular polymers(EPS) that promote the adherence of additional organisms.[7] Acid-producing bacterial species, such as Veillonella, Scardovia, Lactobacillus, and Propioni bacterium, also colonize the bio film, enhancing its cariogenic potential. EPS components not only provide new binding sites for microorganisms but also offer protection and mechanical stability, making the bio film resistant to antimicrobial treatment.[8] The cariogenic potential of Streptococcus mutans bio film, which depends

https://doi.org/10.5281/zenodo.15205084

on acid production, acid resistance, and EPS synthesis, has been well established, with glucosyl transferases (GtfBCD) playing a key role in establishing cariogenic bio films.[9] However, targeting S. mutans and limiting sugar intake alone is insufficient to prevent caries. Other cariogenic species, including Actinomyces, Scardovia, Lactobacillus, and even fungi like Candida albicans, contribute to the biofilm's complexity.[10]

Seaweeds overlook and its significance in dentistry

Seaweeds, also known as marine algae, are multicellular photosynthetic eukaryotes that lack true roots, stems, and leaves and have a thallus structure instead.[11] Marine algae and seaweeds are among the most abundant sources of bioactive compounds, and their significance as a source of novel substances has increased rapidly in recent years.[12] They are utilized in the pharmaceutical industry for the development of drugs to treat various diseases, including cancer, acquired immunodeficiency syndrome (AIDS), inflammation, pain, arthritis, and infections caused by viruses, bacteria, and fungi. They have a wide range of properties ,such as antimicrobial, anti-inflammatory, and antioxidant activities, due to their bioactive compounds.[14]

Zonaria variegata, also known as Lobophora variegata, is a common species of brown seaweed in tropical and subtropical regions. It produces secondary metabolites with diverse bioactive properties, demonstrating immense biomedical potential. It has been used in folk medicine to treat ailments such as eczema, gallstones, gout, fever, menstrual issues, renal problems, and scabies. Compounds derived from Z. variegata exhibit antibiotic, anti-HIV, anticoagulant, anticonvulsant, anti-inflammatory, anti-neoplastic, wound healing, antiulcer, hepato-protective, and antitumor activities. This research aims to investigate the efficacy and safety of Z.variegata extract in dental care solutions, addressing the growing demand for alternatives to synthetic chemicals in dental treatments.[15]

Materials and Methods

Crude Methanol Extract Preparation

Zonaria variegata samples were collected and thoroughly cleaned to remove debris and contaminants. The samples were then dried at 45°C to remove excess moisture and subsequently ground into a fine powder using a mechanical blender. A total of 50grams of the finely powdered seaweed was dissolved in 500ml of methanol and subjected to Soxhlet extraction for efficient solvent extraction of bioactive compounds. The extracted solution underwent distillation, followed by solvent evaporation under reduced pressure to yield the final concentrated extract.

Antimicrobial Activity

The antibacterial activity of the Z. variegata extract was evaluated using the standard well diffusion method against **Streptococcus mutans** and **Shigella sonnei**. The bacterial suspensions, consisting of 24-hour grown strains, were uniformly swabbed onto Luria-Bertani agar (LBA) plates using sterile cotton swabs. Wells were created on the agar plates using a sterile gel puncture tool, and the Z. variegata extract at varying concentrations (25 µg/mL, 50µg/mL, 75µg/mL, and 100µg/mL) was loaded into the wells. A control well containing culture supernatant was also included. The plates were incubated at 37°C for 24hours, after which the antimicrobial activity was assessed by measuring the diameter of the inhibition zones around the wells.

In Vitro Bio film Inhibition Assay

The anti-bio film activity of the Z. variegata extract was tested using **S. mutans, S. sonnei, Staphylococcus aureus, and Micrococcus luteus**. Glass squares (1x1 cm) were used as substrates for bio film formation. These squares were placed in 24-well polystyrene plates containing bacterial inoculum and varying concentrations of Z.variegata extract (30,50,80, and 100 µg/mL). The plates were incubated at 37°C for 24 hours to allow bio film formation. Following incubation, the glass squares were washed twice with phosphate-buffered saline (PBS) to remove loosely attached bacteria. The bio films were

https://doi.org/10.5281/zenodo.15205084

stained using 6% crystal violet and observed under an Olympus CX21i LED microscope at 40x magnification. Confocal microscopy was subsequently used for detailed visualization of the bio film structure.

Antioxidant Assay Using DPPH Radical Scavenging Method

The radical scavenging activity of Z. variegata extract was assessed using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) method. A seaweed extract solution was prepared by dissolving 10mg of the sample in 10mL of methanol. The working DPPH solution was prepared by diluting a stock solution of 24 mg DPPH in 100 mL of methanol to achieve an absorbance of approximately 0.98 (±0.02) at 517 nm. In each reaction, 3 mL of the DPPH working solution was mixed with 100 µL of the seaweed extract and incubated in the dark for 30 minutes. Absorbance was measured at 517nm using a spectrophotometer and the percentage of radical scavenging activity (% RSA) were calculated.

BSA Denaturation Assay

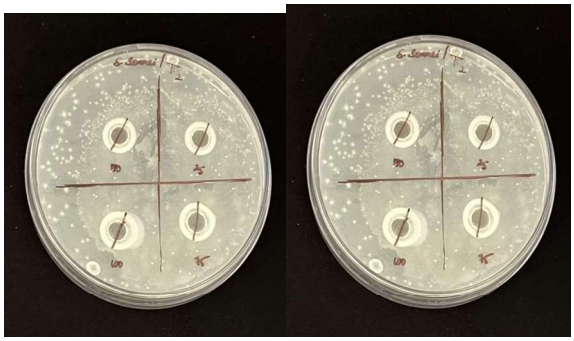
To assess the anti-inflammatory properties of Z. variegata extract, a BSA denaturation assay was performed. A 0.2% (w/v) bovine serum albumin (BSA) stock solution was prepared in Tris-buffered saline and adjusted from pH 8.53 to 6.74 using glacial acetic acid. In separate vials, 500 µL of the BSA solution was mixed with 5 µL of Z. variegata extract dissolved in methanol at two different concentrations (50 ppm and 0.5 ppm).A control sample contained BSA with 5 µL of methanol. The mixtures were heated at 72°C for 5 minutes, followed by cooling for 20minutes. Absorbance was measured at 660nm, and the percentage inhibition of precipitation was calculated relative to the control.

Results

Antimicrobial Activity

The zone of inhibition results for the antimicrobial activity of Z.variegata extract are presented in **Figure1**. The results indicate a concentration-dependent increase in inhibition against both **S.mutans** and **S.sonnei**:

- **S.mutans**: Zones of inhibition for 25, 50, 75, and 100µg/mL were 13mm, 14mm, 15 mm, and 17 mm, respectively.
- **S. sonnei**: Zones of inhibition for the same concentrations were 14 mm, 15 mm, 16mm, and 17 mm, respectively.



(a) (b)

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

Bio film Inhibition

Microscopic analysis of bio films treated with Z. variegata extract (30-100 µg/mL) showed a significant reduction in bacterial bio film formation in a concentration-dependent manner (**Figure 2**). In control samples, bacterial colonies were dense and stratified, while bio film density decreased progressively with increasing extract concentrations.

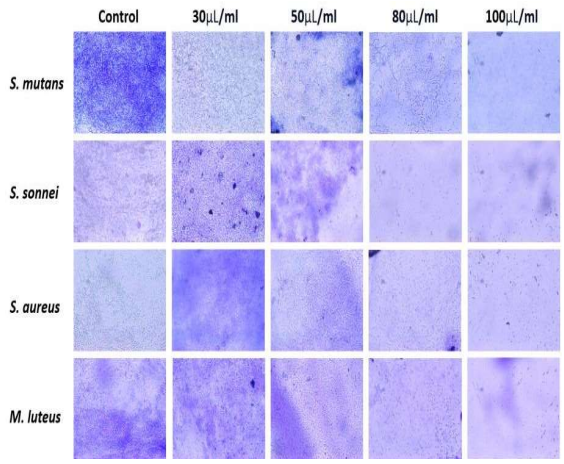


Fig.2: Percentage of Bacterial bio film inhibition of *S.mutans*, *S.sonnei, S.aureus* and *M.luteus* using the antagonistic effect of

synthesized *Z. variegata* extract of concentrations 30 µg/ mL, 50µg/mL, 80µg/mL and 100µg/mL.

Antioxidant Activity

The DPPH radicals cavenging activity of Z. variegate extract at 20, 40, 60, 80, and 100µg/mL showed a dose-dependent increase Ascorbic acid, used as a positive control, exhibited superior scavenging activity compared to the extract; however, the extract demonstrated significant radical neutralization potential.

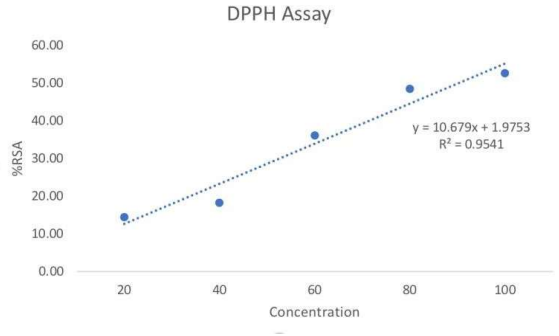


Fig.3: Free radical scavenging activity of *Z. variegata* (20µg/mL, 40µg/mL, 60µg/mL, 80µg/mL and 100µg/mL) on DPPH. Ascorbic acid was used as a positive control.

BSA Denaturation Assay

The inhibition of BSA denaturation by Z. variegata extract was recorded at concentrations of 10-50 µg/mL (**Figure4**). The extract exhibited considerable stabilization of protein structure, suggesting potential anti-inflammatory properties.

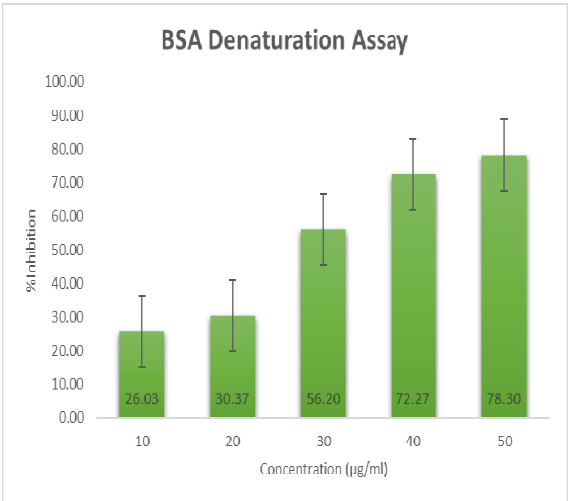


Fig.4: BSAdenaturation assay of *Z. variegata* under different concentrations (10µg/ mL, 20µg/mL, 30µg/mL, 40µg/mL and 50µg/mL)

The antimicrobial activity observed in this study suggests that Z. variegata extract contains bioactive compounds effective against dental pathogens, including **S. mutans** and **S. sonnei**. The increasing zone of inhibition with higher concentrations supports the concentration- dependent efficacy of the extract. These findings align with previous reports on marine algae's antimicrobial properties, particularly their ability to target oral bio films and pathogenic bacteria.

The *in vitro* bio film inhibition assay demonstrated the extract's potential to disrupt and prevent bio film formation. Given that dental bio films contribute significantly to caries development, the findings underscore the promise of **Z. variegata** as an alternative bio film-targeting agent.

The extract also displayed strong antioxidant properties, as evidenced by DPPH scavenging activity. Oxidative stress is a key factor in various oral diseases, including periodontitis and dental caries, highlighting the importance of antioxidant-rich natural products in dental care. Additionally, the BSA denaturation assay results suggest that Z. variegata possesses anti-inflammatory properties, which may be beneficial in managing inflammatory oral conditions.

Conclusion

This study provides substantial evidence supporting the potential application of **Zonaria variegata** extract in dental care. Its antimicrobial, anti-biofilm, antioxidant, and anti- inflammatory properties highlight its promise as a natural alternative to synthetic dental treatments. Future research should focus on identifying the specific bioactive compounds responsible for these effects and conducting in vivo studies to validate these findings

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https://doi.org/10.5281/zenodo.15205084