Antibacterial Potential of Seaweed Chaetomorpha Species Crude Extract Against Oral Pathogen

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ABSTRACT

Introduction: The oral cavity hosts diverse bacterial species, with some contributing to periodontitis—a chronic inflammatory disease affecting tooth-supporting structures. Conventional treatments, including mechanical therapy and systemic antimicrobials, often fall short due to bacterial resistance and side effects. Marine algae, particularly Chaetomorpha species, have shown promising antimicrobial and antioxidant properties, making them potential alternatives for managing oral infections. This study evaluates the antibacterial and antioxidant activity of crude Chaetomorpha extract against common oral pathogens. **Materials and Methods:** Chaetomorpha seaweed was collected from the southeast coast of India, dried, and ground into a fine powder. The crude extract was obtained using ethanol and tested against Streptococcus mutans, Staphylococcus aureus (MRSA), and Klebsiella pneumoniae using the agar diffusion method. Antioxidant activity was assessed using the DPPH free radical scavenging assay, with Vitamin C as a control. Data were analyzed using SPSS software, with statistical significance set at $p<0.05$. **Results:** The antimicrobial assay demonstrated that the zone of inhibition increased with extract concentration for all tested bacterial strains. A statistically significant difference was observed between the extract's high and low concentrations, confirming dose-dependent antibacterial activity. Antioxidant analysis revealed that Chaetomorpha extract exhibited comparable activity to Vitamin C across different concentrations. **Conclusion:** The crude extract of Chaetomorpha species exhibited significant antibacterial activity against oral pathogens, along with strong antioxidant properties. These findings suggest its potential as a natural alternative for managing periodontal infections. Further research, including clinical trials and bioactive compound identification, is necessary to explore its therapeutic applications in oral healthcare.

Keywords: Seaweed, Periodontitis, Antibacterial, oral pathogens, chaetomorpha, eco friendly, innovative technique.

INTRODUCTION :

The oral cavity provides an optimal environment for various types of bacteria to thrive, including Streptococci, Lactobacilli, Staphylococci, Corynebacteria, Porphyromonas gingivalis, and numerous anaerobic species like Bacteroides[1]. This is due to the presence of nutrients, debris from the epithelium, and secretions. Periodontitis, a chronic inflammatory condition affecting the structures supporting teeth, typically begins with the accumulation of plaque biofilm. The bacteria

inhabiting the teeth and gums have evolved alongside their host, creating a delicate balance between pathogenic and beneficial bacteria. Disruption of this balance can lead to an increase in harmful bacteria. According to the "keystone pathogen" theory, certain less abundant microbial pathogens can trigger inflammation by altering the normal microbiota, leading to dysbiosis[2]. Therefore, maintaining this balance through regular plaque control is crucial.

The inflammatory process that ensues in response to dental plaque bacteria is associated with the generation of reactive species and free radicals. Reactive oxygen species like superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$) and reactive nitrogen species like nitroxyl anion, nitrosonium cation, higher oxides of nitrogen, S-nitrosothiols beget a chain reaction that further increases the oxidative stress leading to furthermore tissue destruction[3]. The human body produces intrinsic antioxidants that usually maintain homeostasis in the state of health. During the disease process there is an imbalance between the reactive species and the antioxidants, which further impairs tissue healing[4]. In patients with a hyper-responsive phenotype, adjunctive antioxidant therapies may be necessary along with the elimination of the etiologic agents.

Scaling and root planing is the gold standard treatment for arresting periodontal infections and also for control of dental caries lesions. Mechanical therapy alone is not sufficient for the control of severe periodontal infection as some perio pathogens exhibit the ability to invade gingival epithelial cells[5]. These intracellular organisms act as a constant reservoir for recolonisation of periodontal pockets. Such cases warrant the adjunctive use of systemic and local antimicrobials. In recent times, excessive use of antimicrobials has led to the emergence of resistant bacterial strains[6]. The systemic side effects, taste disturbances, allergic stomatitis associated with the profligate use of systemic antimicrobials also has drawn attention towards the search of alternative natural sources of antimicrobials. Also, antioxidant therapeutics are also being explored as an adjunctive treatment modality in the management of severe cases of periodontitis. Antioxidant supplements in the form of tablets, mouthwashes and lozenges are currently being employed. A therapeutic biomaterial with the properties of both antimicrobial and antioxidant activity would be highly valuable in the management of such cases.

Recent studies have explored the pharmacological properties of various marine organisms like algae, sponges and corals that open avenues for various therapeutic options[7]. Marine algae have been further classified into red (Rhodophyceae), brown (Phaeophyceae), and green algae (Chlorophyceae) based on their pigments. They produce phytochemicals and bioactive compounds like polyunsaturated fatty acids, terpenoids, xanthophylls, carotenoids, phycobilins, chlorophylls, polysaccharides, vitamins, sterols, tocopherol, and phycocyanins that have shown to possess antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory and anti-cancer properties[8].

One such green algae of Cladophoraceae family commonly known as sea emerald or spaghetti seaweed is widespread in the Mandapam coastal region of the Gulf of Mannar on the southeast coast of India. (Class: Ulvophyceae; Order: Cladoporales; Family: Cladoporaceae). It is a photosynthetic powerhouse with the ability to absorb excess trace metals, oxygenate sediments, annihilate nitrogenous wastes and suppress pathogenic bacterial growth. Cheatomorpha

supersaturated the environment with oxygen, shocking the micro-organisms. It also produces chloroform based metabolites that have proven to be toxic to several bacteria. Hence, in our study we explored the antimicrobial activity of crude extract of cheatomorpha species against oral pathogens.

MATERIALS AND METHODS:

The study was conducted at the Department of Biomaterials at Saveetha Dental College.

Sample Collection:

The green seaweed Chetomorphs was collected from the intertidal zone of the southeast Indian coast town of Kanyakumari. After thoroughly washing the plants in tap water to remove any sand or attached fauna, the excess water was drained using blotting paper. The plants were then shade-dried for five days and oven-dried at 60 °C until a constant weight was achieved. Finally, they were ground into a fine powder using a mortar and pestle.

Preparation of extracts:

For obtaining the crude extract of Chetomorpha, 100 gms of powdered Chetomorpha seaweed sample was mixed with 300 ml of 70% ethanol and kept in a shaker for two days, which allowed for the dissolution of the seaweed into the solvent. The resultant solution was filtered and transferred to a beaker which was then left to evaporate to obtain the crude extract. Further, the crude sample was stored in the refrigerator for antibacterial analysis.

Antibacterial Activity:

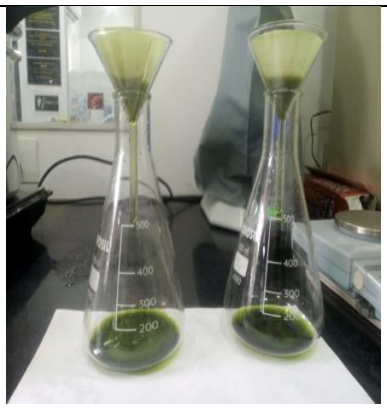
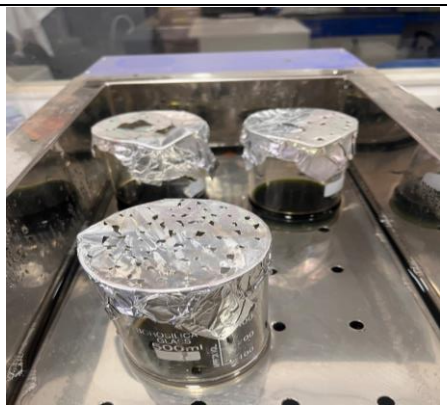
The experiment utilized Staphylococcus aureus (MRSA), Klebsiella pneumoniae, and Streptococcus mutans for assessing the antibacterial activity of the Chetomorpha extract. The antibacterial properties of Chetomorpha seaweed were investigated using the agar diffusion method. The crude Chetomorpha extract was diluted with ethanol to concentrations of 150, 100, and 75 mg/mL, and discs of 5mm diameter were prepared using Whatman filter paper. These discs were sterilized at 160 degrees Celsius in a hot air oven. Methanol was used as a solvent for extraction and served as the control, while a Tetracycline (10mg) disc was used as the standard. After incubating the agar plates at 37 degrees Celsius for 2 days, any clear inhibition zones around the discs were examined and recorded for analysis.

Antioxidant activity:

The antioxidant capability of the crude extract was further examined by evaluating its ability to neutralize the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Different concentrations of the crude extract (100 microliters each) were mixed with 2900 microliters of DPPH solution (120 micromolar) in methanol. The resulting mixture was then kept in the dark at 37°C for 30 minutes, after which the absorbance was measured at 517 nanometers. The percentage inhibition

of the DPPH free radical by the crude extract was calculated for each concentration. Vitamin C was employed as a positive control, and all experiments were conducted in triplicate.




STATISTICAL ANALYSIS - T TEST was done using SPSS software

RESULTS :

ANTIMICROBIAL ANALYSIS

STRAIN	CONCENTRATION	N	MEAN (ZONE OF INHIBITION)	STANDARD DEVIATION	P VALUE

STREPTOCOCCUS MUTANS	ANTIBIOTIC STANDARD	5	21.80	1.483	0.109
	LOW CONCENTRATION - 75	5	19.40	1.673	0.008**
	HIGH CONCENTRATION - 150	5	23.40	1.949	0.336
staphylococcus aureus (MRSA)	ANTIBIOTIC STANDARD	5	17.20	1.923	0.471
	LOW CONCENTRATION - 75	5	16	1.224	0.003**
	HIGH CONCENTRATION	5	20.20	1.483	0.027**
Klebsiella sp.	ANTIBIOTIC STANDARD	5	22.80	1.923	0.021**
	LOW CONCENTRATION - 75	5	19.20	1.303	0.028**
	HIGH CONCENTRATION	5	22.60	2.073	0.983

Antimicrobial assay was performed against common oral pathogens streptococcus mutans, staphylococcus aureus and Klebsiella pneumonia. Amoxicillin was used as the antibiotic standard. The synthesised crude extract was used in low and high concentrations to assess the antimicrobial activity. The zone of inhibition increased with the increase in concentration of the extract for all the bacterial strains tested. On statistical analysis, a significant difference in the mean zone of inhibition was observed between low concentration and antibiotic standard for S. mutans. For S. aureus a statistically significant difference in the mean zone of inhibition was observed between low concentration and high concentration and between high concentration and antibiotic standard. For K. pneumonia, a statistically significant difference in the mean zone of inhibition was observed between low concentration of the extract and antibiotic standard and between low concentration

and high concentration of the synthesized extracts. The results prove that the synthesized extract of chaetomorpha exhibits good antimicrobial activity against the tested pathogens.

Antioxidant analysis :

The mean antioxidants activity increased with the increase in concentration of the extract. Vitamin C was used as the control. On statistical analysis, no significant difference in the mean antioxidant activity was observed on comparing the synthesized extract with the control at all concentrations. The results prove that the chaetomorpha extract was comparable to antioxidant activity as Vitamin C.

CONCENTRATION	SAMPLE	N	MEAN % OF ANTIOXIDANT ACTIVITY	STANDARD DEVIATION	P VALUE
40	CRUDE	5	28.60	2.302	0.763
	VIT C	5	20.60	2.408	
60	CRUDE	5	40.60	3.847	0.143
	VIT C	5	33.80	2.167	
80	CRUDE	5	46.80	2.387	0.552
	VIT C	5	37.80	1.788	
100	CRUDE	5	60.00	1.581	0.373
	VIT C	5	51.00	1.000	
120	CRUDE	5	70.00	1.581	0.486
	VIT C	5	62.00	2.000	

DISCUSSION:

The oral cavity hosts a diverse range of bacteria, from beneficial to harmful species, thriving amidst nutrients and epithelial debris. Disruption of this delicate microbial balance, often initiated by plaque biofilm accumulation, can lead to periodontitis, a chronic inflammatory condition affecting tooth-supporting structures. This imbalance can trigger inflammation by altering the normal microbiota, necessitating regular plaque control to maintain equilibrium. The ensuing inflammatory response generates reactive oxygen and nitrogen species, overwhelming intrinsic antioxidant defenses and impeding tissue healing. In severe cases, adjunctive antioxidant therapies alongside elimination of etiologic agents may be necessary. While scaling and root planing remain the gold standard for treatment, severe infections may require adjunctive antimicrobials; however,

excessive use has led to the emergence of resistant strains, prompting exploration of alternative natural antimicrobials supplements. Use of natural antioxidant supplements further augment in the restoration of the tissue back to a state of health. Hence, the development of therapeutic biomaterials with combined antimicrobial and antioxidant properties holds promise for effective periodontal management.

In the present study, we synthesized crude extract from seaweed chaetomorpha and assessed the antimicrobial and antioxidant activity. Antimicrobial assay was performed against common oral pathogens s. Mutans, s. Aureus and K. pneumonia using disc diffusion method with amoxicillin used as the antibiotic standard. The developed extract showed good antimicrobial activity against the tested microorganisms. On analysis of the antioxidant activity, chaetomorpha extract exhibited comparable antioxidant activity to that observed with Vitamin C. These properties may be attributed to the presence of bioactive compounds like flavonoids, tannins, phenols in the extract. The results of our study are in accordance with other similar studies that assessed the antimicrobial and antioxidant activities of chaetomorpha species. A study by Thanigaivel et al showed good antimicrobial activity of Chaetomorpha antennina against V. parahaemolyticus. They stated that this property may be attributed to the 16 major bioactive compounds that were identified in the extract. They also stated that C. antennina plays a noteworthy role in scavenging free radicals, such as ABTS, OH radicals, hydroxyl peroxides, and DPPH, as well as in chelating effects and overall antioxidant activity. Another study by Jebamalar et al. showed good antioxidant activity similar to our results and they stated that antioxidants sourced from seaweeds offer distinct advantages over synthetic counterparts. Their natural origin renders them more advantageous compared to artificial alternatives due to their lack of side effects, contrasting with synthetic antioxidants which have been associated with genotoxic effects. The initial phytochemical analysis of the dichloromethane crude extract from the Chetomorpha species revealed the existence of alkaloids, flavonoids, tannins, glycerides, terpenoids, phenols, and sterols. Previous studies by Sivakumar and Safhi, Subathraa and Poonguzhali, as well as Abhishek et al., also documented the presence of a variety of phytochemicals in the crude extracts of Chetomorpha species, including carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, terpenoids, glycerides, phenols, and sterols.

Chaetomorpha species are a genus of green macroalgae commonly found in marine environments, particularly in shallow coastal waters and estuaries worldwide. They are characterized by their filamentous, bushy appearance and are known for their ecological significance, playing roles in nutrient cycling, habitat provision, and carbon sequestration. Research on Chaetomorpha The species has revealed promising anti-inflammatory and antioxidant properties, making them potential candidates for therapeutic applications. Studies have shown that extracts from Chaetomorpha species possess significant anti-inflammatory activity by inhibiting pro-inflammatory mediators and cytokines, thereby reducing inflammation-related tissue damage. Additionally, these extracts exhibit strong antioxidant activity, scavenging reactive oxygen and

nitrogen species and preventing oxidative stress-induced cellular damage. The presence of bioactive compounds such as polyphenols, flavonoids, and carotenoids in Chaetomorpha extracts have shown to contribute to their anti-inflammatory and antioxidant effects. These findings suggest that Chaetomorpha species hold potential as natural sources of compounds for developing anti-inflammatory and antioxidant therapies to combat various inflammatory and oxidative stress-related diseases like periodontitis.

Various researchers have explored marine algae based local drug delivery agents for the treatment of periodontal diseases. Mahendra et al developed spirulina based local drug delivery in gel format for the treatment of periodontal pockets. They concluded that spirulina gel application caused a decrease in probing pocket depth along with clinical attachment level gain. They attributed the clinical improvement observed to the anti-inflammatory and antioxidant effects of spirulina due to the inhibition of ROS and cytokine release through inactivation of the nuclear factor kappa pathway. They stated that Phycocyanin was the major compound that was responsible for the biologic effects of spirulina. Similar to such exploration of the pharmaceutical applications of seaweeds, the synthesised crude extract of chaetomorpha in this study also proved potential antimicrobial and antioxidant activity. Further cell lines, animal trials and human clinical trials with long term follow up should be conducted to analyse the clinical application and efficacy of the chaetomorpha extract. Drug screening and toxicity analysis should also be performed to assess the principal bioactive compounds present in the extract. This would help us understand the exact mechanism of action for the antimicrobial and antioxidant properties allowing for targeted drug delivery and personalised care for the treatment of periodontal disease.

CONCLUSION:

One of the main sources of biomass in the marine environment, marine algae are also a potential source of novel, diverse, and distinctive chemicals. Alginates, carrageenan, and agar are just a few of the chemicals derived from seaweed that have been utilized for years in traditional medicine, pharmacology, and gastronomy. Other substances have antimicrobial, bacteriostatic, antiviral, anticancer, anti-inflammatory, and antifouling properties. As a result, seaweed may offer intriguing bioactives that can be used to treat human diseases or new antimicrobial agents that can take the place of synthetic antibacterials used in food production and agriculture with SPSS statistical software analysis the P value stated to be <0~~2~~.05

REFERENCES:

1. Li X, Liu Y, Yang X, et al. The Oral Microbiota: Community Composition, Influencing Factors, Pathogenesis, and Interventions. *Front Microbiol* 2022; 13: 895537.
2. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. *Nature Reviews Microbiology* 2012; 10: 717–725.
3. Rutkowski R, Pancewicz SA, Rutkowski K, et al. [Reactive oxygen and nitrogen species in inflammatory process]. *Pol Merkur Lekarski* 2007; 23: 131–136.
4. Website. DOI: 10.1155/2021/6204096.
5. Dickinson BC, Moffatt CE, Hagerty D, et al. Interaction of oral bacteria with gingival epithelial cell multilayers. *Molecular Oral Microbiology* 2011; 26: 210–220.

6. Emergence of antibiotic resistance due to the excessive use of antibiotics in medicines and feed additives: A global scenario with emphasis on the Indian perspective. *Emerging Contaminants* 2024; 10: 100389.
7. Exploring the potential of marine natural products in drug development: A comprehensive review. *Phytochemistry Letters* 2024; 59: 124–135.
8. El-Beltagi HS, Mohamed AA, Mohamed HI, et al. Phytochemical and Potential Properties of Seaweeds and Their Recent Applications: A Review. *Marine Drugs* 2022; 20: 342.
9. Stabili L, Quarta E, Giotta L. The seaweed Chaetomorpha linum cultivated in an integrated multitrophic aquaculture system: A new tool for microplastic bioremediation? *Sci Total Environ* 2024; 954: 176262.
10. Sattanathan G, Padmapriya S, Almanaa TN, et al. Impact of Chaetomorpha aerea-enriched diet on growth, feed utilization, and haemato-immunological responses in Clarias batrachus challenged with Aeromonas hydrophila. *Microb Pathog* 2024; 196: 106962.
11. Hirschfeld J, Chapple ILC. *Periodontitis and Systemic Diseases: Clinical Evidence and Biological Plausibility*. Quintessenz Verlag, 2021.
12. Armstrong D, Stratton RD. *Oxidative Stress and Antioxidant Protection: The Science of Free Radical Biology and Disease*. John Wiley & Sons, 2016.
13. Souza AF, Fraga MAA, Correr AB, et al. Resin infiltrant with antibacterial activity: effects of incorporation of DMAHDM monomer and NACP on physical and antimicrobial properties. *J Appl Oral Sci* 2024; 32: e20240263.
14. Antioxidant and antibacterial activity of Chaetomorpha antennina against shrimp pathogen Vibrio parahaemolyticus. *Aquaculture* 2014; 433: 467–475.
15. Sivakumar SM, Safhi MM. Isolation and screening of bioactive principle from Chaetomorpha antennina against certain bacterial strains. *Saudi Pharm J* 2013; 21: 119–121.
16. Pereira L. *Therapeutic and Nutritional Uses of Algae*. CRC Press, 2018.
17. Aravind J, Kamaraj M, Prashanthi Devi M, et al. *Strategies and Tools for Pollutant Mitigation: Avenues to a Cleaner Environment*. Springer Nature, 2021.
18. Sattanathan G, Padmapriya S, Kadaikunnan S, et al. Marine macroalgae Chaetomorpha aerea as a dietary supplement: Optimizing immunity and resistance to Edwardsiella tarda in tilapia (Oreochromis mossambicus). *Fish Shellfish Immunol* 2024; 154: 109956.
19. Geng Y, Xing R, Zhang H, et al. Inhibitory effect and mechanism of algicidal bacteria on Chaetomorpha valida. *Sci Total Environ* 2024; 914: 169850.
20. Ortiz-Barragán E, Estrada-Soto S, Giacoman-Martínez A, et al. Antihyperglycemic and Hypolipidemic Activities of Flavonoids Isolated from Smilax Dominguensis Mediated by Peroxisome Proliferator-Activated Receptors. *Pharmaceuticals (Basel)*; 17. Epub ahead of print 30 October 2024. DOI: 10.3390/ph17111451.
21. JCDR - Spirulina, Local drug delivery, Green algae, Periodontitis, https://doi.org/10.7860%2FJCDR%2F2013%2F5793.3517 (accessed 2 April 2024).
22. Arrari F, Ortiz-Flores R-M, Lhamyani S, et al. Protective Effects of Spirulina Against Lipid Micelles and Lipopolysaccharide-Induced Intestinal Epithelium Disruption in Caco-2 Cells: In Silico Molecular Docking Analysis of Phycocyanobilin. *Nutrients*; 16. Epub ahead of print 27 November 2024. DOI: 10.3390/nu16234074.
23. Tavares J, Oliveira AV, de Souza Nascimento T, et al. Aqueous extract of Spirulina exerts

neuroprotection in an experimental model of Alzheimer sporadic disease in mice induced by Streptozotocin. *Metab Brain Dis* 2024; 40: 26.