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# Preparation of Crude Extract from Chaetomorpha Species Seaweed and Analysis of their Antioxidant Property - An in Vitro Study

# Running Title: Antioxidant Property of Chaetomorpha Species Seaweed

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**Keywords:**
Chronic periodontitis; Seaweed; Marine algae; Antioxidants; Chaetomorpha species; Periodontal therapy.

## Abstract

INTRODUCTION: Chronic periodontitis is a leading cause of tooth loss leading to poor quality of life worldwide. Dental plaque biofilm is the primary etiologic factor for the development of periodontal infections. The ensuing interaction between the plaque microflora and the host results in cascades of events ultimately leading to an inflammatory response in the periodontal tissues. Antioxidants are molecules that fight free radicals in our body. Chaetomorpha species, a common and widespread green seaweed genus with unbranched filaments. Its wide variety of bioactive substances makes it a potential candidate to explore its antioxidant properties. AIM: To analyze the antioxidant property of crude extract prepared from chaetomorpha species MATERIALS AND METHODS: The study was conducted at the Department of Biomaterials, Saveetha Dental College. Cheatomorpha species seaweed was collected from the Bay of Bengal ocean along the Chennai coastline and ethanolic crude extract was prepared. The extract was then analyzed for antioxidant activity using DPPH assay, H2O2 radical scavenging test and total antioxidant analysis. Control used was ascorbic acid. RESULTS: Antioxidant activity gradually increased in accordance to the increase in concentration of the sample. The highest concentration of 100ug/ml showed 64% of antioxidant activity whereas ascorbic acid which is taken as control showed 80% of antioxidant activity at 100ug/ml concentration. CONCLUSION: Natural resources are often used for discovery of drugs and play a vital role in programs of drug development. The biological activities of marine algae are increasing tremendously. Therefore, mechanical therapy alone is not sufficient in the treatment of periodontal infections. In severe periodontitis, adjunct therapy in the form of antioxidant supplements would help to overcome the tissue destruction mediated oxidative stress and bring the tissues back to homeostasis.

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## 1. Introduction

Periodontal disease is a chronic inflammatory condition affecting the supporting tissues of the teeth leading to clinical attachment loss, bone and tooth loss. According to a recent systematic review and meta-analysis on the prevalence of periodontal disease among Indian adults, overall prevalence of periodontal disease was found to be 51% and gingivitis was found to be 46.6%. With an increase in lifestyle modifications and emergence of chronic diseases, periodontal infections are at a rise among the global population (1) .

The primary etiologic factor is the dental plaque biofilm causing the development of periodontal infections(2). The ensuing interaction between the plaque microflora and the host results in cascades of events ultimately leading to an inflammatory response in the periodontal tissues (3). PMNs the 1st line of defense and other inflammatory cells like macrophages are recruited by the host to manage the microbial attack. These cells control the microbes through two pathways i.e. oxidative and non-oxidative killing. It is during the oxidative burst killing method employed for microbial killing, that there is an increase in oxygen consumption, activation of HMP shunt and generation of free radicals and ROS (4).

ROS are molecules such as hydrogen peroxide (H2O2), hypochlorous acid (HOCI) and singlet oxygen (1O2) that are required for normal biologic processes. Free radicals are defined as the atomic species or molecular species of one or more unpaired electrons and are of independent existence (5). They are a group of highly reactive species, oxidizing a wide range of biomolecules that are significant to cell and tissue function. They are produced both endogenous and exogenous. Endogenously they are produced as by-products of metabolic pathways, phagocytes and cells of connective tissue. Exogenously they are produced from trauma, heat, radiation, UV light, smoking, infection, drugs, pollutants in air, ozone, and chemicals from industries (6).

Though they have extremely short half-lives but they cause substantial tissue damage through various mechanisms like causing protein and DNA damage, peroxidation of lipids, enzyme oxidation, leading to initiation of inflammatory cascades.

Antioxidants are molecules that fight free radicals in our body. Any substance that, when present in low concentrations compared to those of an oxidisable substrate, considerably slows down or stops that substrate from oxidizing is an antioxidant (7). Our body has its own antioxidant defenses to keep free radicals in check. The imbalance in ROS and the antioxidant system leads to chronic diseases, cardiac diseases and cancer. In periodontal disease, there is an increase in the production of ROS and FR due to the increased microbial killing through oxidative mechanisms(8). This leads to increased tissue destruction through ground substance degradation, collagenolytic, inflammatory cytokine release activation, activation of NFKB pathway(9). To counteract the increased tissue destruction due to ROS and FR release, adjunctive therapy in the form of antioxidant supplements is warranted along with gold standard non-surgical and surgical periodontal therapy. Antioxidants can be classified based on their source as endogenous antioxidants and dietary antioxidants. They are also classified according to their mode of action, location and solubility. They are found in food, especially in fruits, vegetables, and other plant-based, whole foods (10). Several vitamins, such as vitamins E and C, are effective antioxidants. Antioxidants prevent free radical formation, detoxify ROS and modify them and form them as less reactive species and also repairs the damage caused by ROS (11).

Seaweeds are marine multicellular algae and possess a large amount of bioactive compounds. Seaweeds are plentiful in minerals, vitamins, and polysaccharides and they are a potential source of bioactive substances with antioxidant, antimicrobial and anti-inflammatory properties (12). Algae contains substances such as alkaloids, flavonoids, phenols, tannins, terpenoids, pigments, glycosides, and steroids which plays a role in defense mechanisms (13). They are useful in the commercial application of pharmaceuticals, cosmetics, medical, food industry and agriculture. In recent years marine algae have attracted the attention of scientists in the world to develop new drugs from natural bioactive compounds (14).

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One such species of marine algae are the Chaetomorpha species, a common and widespread green seaweed genus with unbranched filaments. Its wide variety of bioactive substances makes it a potential candidate to explore its antioxidant properties (15). In our study we have prepared the crude extract from seaweed Chaetomorpha species and analyzed their antioxidant properties.

Our team has extensive knowledge and research experience that has translate into high quality publications (16–25)

## 2. Materials and Methods

The study was conducted at the Department of Biomaterials, Saveetha Dental College. Cheatomorpho species seaweed was collected from the Bay of Bengal ocean along the Chennai coastline. The sample was manually collected; epiphytes and the debris was removed by washing in running tap water and washed again with distilled water.

PREPARATION OF ETHANOLIC CRUDE EXTRACT

The sample was scrubbed and cleaned implementing distilled water along with was contained for drying within a hot oven of air at the temperature of 60 deg Celsius. After drying, the sample was then crushed into a coarse powder using mortar and pestle. Sample's 20g was attached to 200 ml of ethanol's 70% in the middle of a flask of conical as well as the sample moved within a shaker for some days duration, and the duration is 2 days. The extract was filtered using Whitman filter paper. The filtrate samples were placed in a water bath below 60 deg Celsius and crude concentrated extract was obtained.

**Figure:1** represents the chaetomorpha sample


**Figure 2** represents the dissolved sample in 70% of ethanol Fig:3 represents the sample placed in orbital shaker

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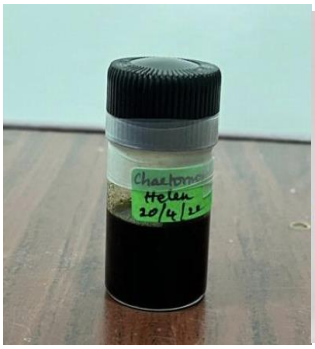


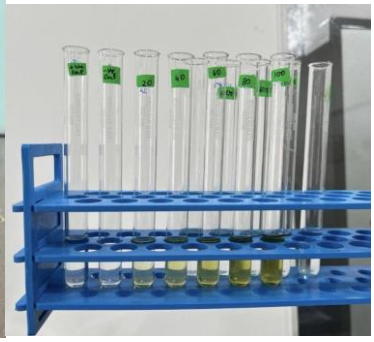

**Figure:4** represents the crude chaetomorpha sp extract **Figure:5** represents the sample at different concentrations

DPPH Assay

The potential of antioxidants synthesized of nanoparticles from determined and seaweed on the top of basis about activity of scavenging about stadle 1, in addition, "*1-diphenyl-2-picryl budazxal"* free radical depended above the process of Daun et al. (2006), Raisshkumar et al. 2021; Sivaperumal et al.(2015) In the place of various 100 ul concentrations about the crude subtract were combined by 2900 ul "diphenylpicrylhydrazyl(DPPH)" blend in the middle of ethanol along with incubated within darkness at the temperature of 37 degree celsius for half hour duration. The absorbance was noted at 517 nanometers.

Free radical inhibition through DPPH in 1% percentage was measured by the maintaining equation:

"Inhibition percentage (1%) = (A blank - A sample)/ A blank * 100"

A blank is the control absorbance reaction along with a specimen is the test compound absorbance. The inhibition values were measured on behalf of the several concentrations about the synthesised of nanoparticles deriving out of seaweed. Acid regarding ascorbic implied as positive handles (Liu et al. 2007) and Along with that all the experiments were moved out in triplicate.

Scavenging of hydrogen peroxide (H202)

Ability of the nanoparticles synthesized from the seaweed to scavenge H2O2 was determined according to the method of Ruch et al. (1989) with the slight modification suggested by Rajeshkumar et al., 2021 and Kamala et al 2015 where 40mM H2O was prepared and the concentration was determined spectrophotometrically by measuring the absorption with the extraction coefficient for H2O2 of 81 M-1 cm1. The different concentration of crude extract and standard (ascorbic acid) were added to 0.6 ml of 40mM H202 solution and the absorbance of HaO2 was determined at 230 nm after 10 min incubation against a blank solution, containing phosphate buffer without hydrogen peroxide.

The percentage of scavenging of H2O2 was calculated as follows

Scavenging effect (%) = (A cont -A test) /A cont * 100

Total antioxidant activity

Total activity of antioxidants about nanoparticles synthesized deriving out of the seaweed was selected as per the process of prieto et al,(1999); Kamala et al.(2015) along with Sivaperumal et al. (2015) by straight moderation. In depth, specimens' 0.3 ml were completed in several in various concentrations by reagent solution 3ml ("28mM sodium phosphate", "0.6 m sulfuric acid", along with "4mM ammonium molybdate"), and combination of reaction was incubated at the temperature of 95 degree Celsius and the duration time was 90 minutes in the middle of water bath. All sample's absorbance combinations were calculated at 695 nm.

All activity of antioxidant has been explored in the amount of percentage maintaining the below mentioned formula,

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"Activity percentage (%6) = (a blank - a sample) / a blank*100".

## 3. Results

DPPH RADICAL SCAVENGING (%)

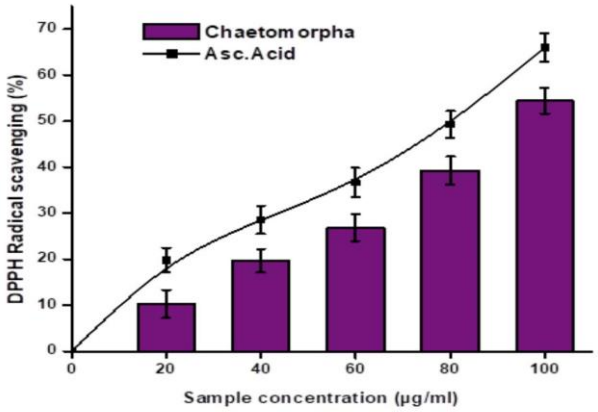


**Figure:6** represents the DPPH radical scavenging activity of chaetomorpha and ascorbic acid

| Concentration | AA% | Standard Error | Chaetomorpha | Standard error |
|---|---|---|---|---|
| 20 | 18.79 | 2.6 | 10.28 | 3 |
| 40 | 25.53 | 3 | 19.64 | 2.5 |
| 60 | 36.61 | 3.2 | 26.84 | 2.9 |
| 80 | 49.26 | 2.9 | 39.27 | 3 |
| 100 | 65.97 | 3.1 | 54.39 | 2.8 |

H2O2 SCAVENGING (%)

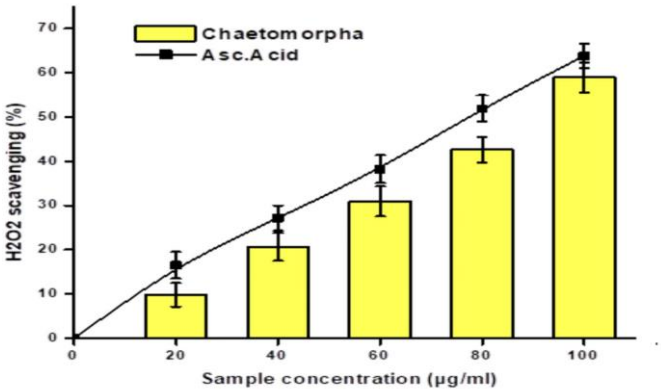


**Figure: 7** represents the H2O2 radical scavenging activity of chaetomorpha and ascorbic acid

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| Concentration | AA% | Standard error | Chaetomorpha | Standard error |
|---|---|---|---|---|
| 20 | 16.53 | 3 | 9.85 | 2.7 |
| 40 | 27.16 | 2.8 | 20.67 | 3.1 |
| 60 | 38.24 | 3.1 | 30.94 | 3.4 |
| 80 | 51.92 | 3 | 42.65 | 2.9 |
| 100 | 63.85 | 2.8 | 58.96 | 3.4 |

TOTAL ANTIOXIDANT ACTIVITY

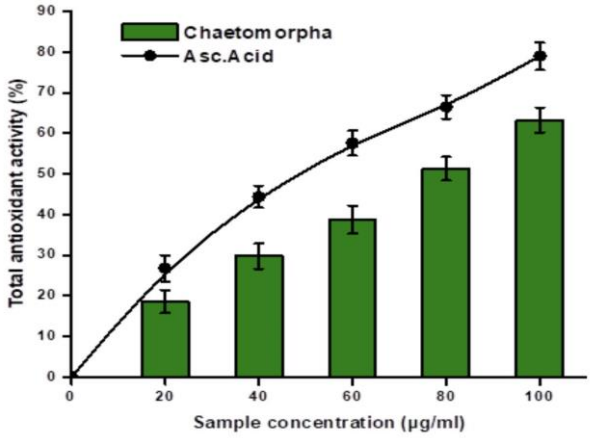


**Figure: 8** represents the Total antioxidant activity of chaetomorpha and ascorbic acid

| Concentration | AA% | Standard error | Chaetomorpha | Standard error |
|---|---|---|---|---|
| 20 | 26.77 | 3.2 | 18.58 | 2.8 |
| 40 | 44.38 | 2.7 | 29.77 | 3.1 |
| 60 | 57.59 | 3.1 | 38.75 | 3.4 |
| 80 | 66.46 | 2.9 | 51.32 | 2.9 |
| 100 | 78.98 | 3.4 | 63.25 | 3.1 |

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## 4. Discussion

Periodontitis is an infection driven inflammatory process affecting the supporting tissues of the teeth. The gingival sulcus is a unique space where the gingival soft tissues are in direct contact with the tooth structure through junctional epithelium. This space acts as a portal of entry for the periodontal microorganisms to enter the systemic circulation resulting in a chronic low grade systemic inflammatory condition. With increase in microbial load or hyper-responsive host response there is increase in microbe and host mediated tissue destruction through release of proinflammatory mediators. Through PMN mediated microbial killing, there is an ensuing respiratory burst phenomenon with production of free radicals and reactive oxygen species. These molecular species with short half lifes cause substantial tissue damage by initiating reactive chain reactions. They mediate tissue damage through various mechanisms affecting normal cellular and molecular functioning ultimately initiating inflammatory reactions. These reactive molecular species also affect multiple systems of the body. Indeed, studies have proven a potential pathobiologic link between periodontitis and cardiovascular diseases, metabolic disorders like diabetes mellitus, adverse pregnancy outcomes, respiratory condition, Alzheimer's disease etc.

The human body has inherent compensatory mechanism to counteract the oxidative stree through production of antioxidants. These processes include internal production of antioxidants as well as external supply from diet or supplements. Examples of various endogenous antioxidants are superoxide dismutase, glutathione peroxidase, catalase, reduced glutathione etc. Additional exogenous dietary antioxidant supplements can also be employed as adjunct therapy in the treatment of periodontal conditions. Though various food substances are rich sources of antioxidants like vit c, vit a, carotenoids, flavonoids, recent research has explored the antioxidant potential of marine seaweeds. Diverse organic and inorganic compounds found in the marine environment are good sources of significant amounts of lipids, proteins, vitamins, and minerals. Seaweeds and sponges are the only food group that offers greater defense against radiation and other environmental toxins. Sulfated polysaccharides are a diverse class of macromolecules with a variety of crucial biological functions. Tmarine algae are rich sources of sulfated polysaccharides. Important pharmacological properties of sulfated polysaccharides from algae include anticoagulant, antioxidant, and anti-inflammatory, antiviral, antibacterial, anti-proliferative, anti-tumor, anti-complementary, and anti-adhesive activity. They have been shown to be crucial antioxidants and free radical scavengers for preventing oxidative damage in living organisms. Several potent medicinal metabolites have been found in a variety of marine seaweed, including Eisenia bicyclis, Sargassum sp., and Padina sp.

Mechanical therapy alone is not sufficient in the treatment of periodontal infections. In severe periodontitis, adjunct therapy in the form of antioxidant supplements would help to overcome the tissue destruction mediated oxidative stress and bring the tissues back to homeostasis. Dietary antioxidant supplements, antioxidant mouthwashes and lozenges have been formulated and applied for the same. In the present study, we have prepared a crude extract of chaetomorpha species seaweed and analysed its antioxidant activity.

To assess the antioxidant capacity of Chaetomorpha sp., a radical scavenging test was carried out. Higher scavenging capacity is indicated by a lower IC50. By using the DPPH radical quenching experiment, extracts of Chaetomorpha sp. demonstrated significant antioxidant activity in a dose-dependent manner and it was comparable to the antioxidant activity shown by control ascorbic acid samples. Antioxidant activity was increased in the ethanolic extract of Chaetomorpha sp. At 100 ug/ml, which has a high concentration of phenolic components. This increase in radical scavenging activity may be attributed to the hydroxyl groups in the phenolic compounds. Similarly, with H2O2 radical scavenging analysis, the scavenging activity increased in accordance with the increase in concentration of the sample. Also, the scavenging activity was comparable to that expressed by control ascorbic acid.

Antioxidant activity gradually increased in accordance to the increase in concentration of the sample. The highest concentration of 100ug/ml showed 64% of antioxidant activity whereas

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ascorbic acid which is taken as control showed 80% of antioxidant activity at 100ug/ml concentration.

In comparison to ascorbic acid, the ethanol extract of Chaetomorpha exhibited comparable antioxidant activity proving its potential as an antioxidant source. Further invitro and in vivo tests can be conducted to further analyse its antioxidant activity in biologic systems and to develop the best possible route of delivery for periodontal applications.

## 5. Conclusion

Natural resources are often used for discovery of drugs and play a vital role in programs of drug development. The biological activities of marine algae are increasing tremendously. There are strong correlations between oxidant state and periodontal status, and that oxidative stress may be a key contributor to the pathophysiology of periodontitis and the resulting tissue damage. Therefore, mechanical therapy alone is not sufficient in the treatment of periodontal infections. In severe periodontitis, adjunct therapy in the form of antioxidant supplements would help to overcome the tissue destruction mediated oxidative stress and bring the tissues back to homeostasis. Antioxidant supplementation should be taken into consideration to enhance the efficacy of various surgical and non-surgical periodontal therapies. Dietary antioxidant supplements, antioxidant mouthwashes and lozenges have been formulated and applied for the same. Well planned and designed dosage should be done by doctors for its effective management in treatment of periodontal therapies.

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Conflict of Interest

The author declares that there were no conflicts of interests in the present study.

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