EXPLORING ANTIOXIDANT ACTIVITY IN *SARGASSUM, ULVA, AND GRACILLARIA* SPECIES: A PHOSPHOMOLYBDENUM ASSAY APPROACH

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Abstract

This study evaluated the antioxidant potential of extracts from three different seaweed species, namely *Ulva lactuca*, *Sargassum wightii*, and *Gracillaria edulis*, using the Phosphomolybdenum assay. Mean optical density (OD) values obtained from the assay indicated varying levels of antioxidant activity among the seaweed extracts. Among these seaweeds, *Gracillaria edulis* extracts exhibited the highest antioxidant activity, with a maximum mean OD value of 1.9±0.0005 observed at a concentration of 80 µg/mL, followed by 1.86±0.066 at 60 µg/mL concentration. *Sargassum wightii* displayed moderate antioxidant activity, ranging from 1.5 to 1.78, while *Ulva lactuca* extracts exhibited the lowest antioxidant potential, ranging from 0.80 to 0.96. Importantly, increasing the concentration of seaweed extracts significantly enhanced their antioxidant potential. The elevated OD values suggest the ability of the seaweed extracts to effectively reduce ferric ions, indicating their antioxidant activity. Overall, *Gracillaria edulis* extracts showed superior antioxidant activity compared to *Sargassum wightii* and *Ulva lactuca* extracts, highlighting their potential as valuable sources of natural antioxidants.

Keywords: *Sargassum wightii, Ulva lactuca, Gracillaria edulis*, antioxidant.

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Introduction:

Marine macroalgae, commonly known as seaweeds, are rich sources of bioactive compounds with diverse pharmacological properties. Among them, species of *Sargassum wightii, Ulva lactuca,* and *Gracillaria edulis* have gained considerable attention due to their potential health benefits attributed to various bioactive compounds such as polysaccharides, polyphenols, pigments, and vitamins. (Mohan *et al*., 2023). Antioxidants are molecules that play a crucial role in neutralizing harmful free radicals and reducing oxidative stress in living organisms (Mamta *et al*., 2014).

Sargassum wightii, a genus of brown algae, is renowned for its rich nutritional profile and bioactive constituents. Research has indicated that *Sargassum wightii* extracts exhibit promising antioxidant activity, making them a subject of interest in pharmaceutical and nutraceutical industries (Antonisami *et al*., 2012*). Ulva lactuca,* commonly known as green seaweed or sea lettuce, is recognized for its high content of essential nutrients, vitamins, and minerals. Studies have revealed that *Ulva lactuca* species possess antioxidant compounds which confer protective effects against oxidative stress-related diseases (Mohan *et al*., 2023).

Gracillaria edulis, a genus of red algae, is valued for its therapeutic properties and nutritional significance contains a wide range of bioactive compounds including sulfated polysaccharides, which exhibit potent antioxidant activity (Pradhan *et al*., 2022).The antioxidant potential of *Sargassum, Ulva,* and *Gracillaria* species has been the subject of numerous scientific investigations. Understanding the antioxidant capacity of these seaweeds is essential not only for exploring their potential health benefits but also for their utilization in various industries (Kalpana *et al*., 2021).

The phosphomolybdenum assay is one of the widely used methods for the estimation of total antioxidant activity in natural products including seaweeds. This assay is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds present in the sample under acidic conditions, forming a green phosphate/Mo (V) complex with a maximum absorbance at 695 nm. The intensity of the green colour is directly proportional to the total antioxidant capacity of the sample (Sharma and mujundar, 2003).

In this study, we aim to evaluate and compare the antioxidant activity of *Sargassum wightii, Ulva lactuca and Gracillaria edulis* using the phosphomolybdenum assay. By quantifying the total antioxidant capacity, we seek to elucidate the potential health-promoting properties of these marine algae and explore their possible applications in functional foods, nutraceuticals, and pharmaceuticals. Understanding the antioxidant potential of these seaweed species can contribute to the development of novel antioxidant-rich products for preventive healthcare and disease management.

Materials and Methods:

The specimens were obtained from the coastal region of Rajakkamangalam Thurai, Kanniyakumari. Following collection, the samples underwent a thorough washing process and were subsequently air-dried under shade for a duration of 7 days. Utilizing the Soxhlet extraction technique, approximately 50 grams of dried powder from *Sargassum wightii* (Brown Algae), *Ulva lactuc*a (Green Algae), and *Gracillaria edulis* (Red Algae) were each combined with 500 mL of autoclaved distilled water for extraction. The resultant extracts were stored at 4°C until further analysis.

PHOSPHOMOLYBDENUM ASSAY (PM)

Total antioxidant activity was estimated by phosphomolybdenum assay.1ml each of 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water.

About 20-100 µl extracted of Samples (*Ulva lactuca, Sargassum wightii* and *Gracillaria edulis*) were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were at normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Percentage of inhibition values from samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

PM assay is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the sample and subsequent formation of a bluish green colored phosphate/Mo (V) complex at acid pH. Use the absorbance readings obtained from the calibration standards to construct a standard curve.

Determine the total antioxidant capacity of the sample extracts by comparing their absorbance values to the standard curve. Express the results as equivalents of the standard antioxidant per unit volume or weight of the sample. Perform appropriate quality control measures, including blank controls and replicates, to ensure the accuracy and precision of the assay results.

Analyze the data to evaluate the total antioxidant capacity of the sample extracts and compare them

to standard antioxidants or other samples. Interpret the results in the context of the experimental objectives and the specific properties of the sample extracts. The phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of algae extracts.

Results and Discussion:

The assessment of antioxidant potential was conducted using the Phosphomolybdenum assay, which determined the absorbance representing the total antioxidant capacity. Table-1 and Figure-1 illustrate the mean OD values obtained from the assay. *Ulva* extracts exhibited mean OD values ranging from 0.80 to 0.96, while *Sargassum* extracts ranged from 1.53 to 1.78, and *Gracillaria* extracts ranged from 1.69 to 1.9.

Among the tested seaweeds, *Gracillaria e*xtracts demonstrated the highest antioxidant activity, with a maximum mean OD value of 1.9±0.0005 observed at a concentration of 80 µg/mL, followed by 1.86±0.066 at 60 µg/mL concentration. *Sargassum* spp. displayed moderate antioxidant activity, ranging from 1.5 to 1.78, whereas Ulva extracts exhibited the lowest antioxidant potential, ranging from 0.80 to 0.96. Notably, an increase in concentration significantly enhanced the antioxidant potential of the seaweed extracts.

The elevated OD values indicate the ability of the plants to effectively reduce ferric ions, highlighting their antioxidant activity. Based on these findings, it can be analysed that *Gracillaria edulis* extracts possess superior antioxidant activity compared to *Sargassum wightii* and *Ulva lactuc*a extracts. The high antioxidant activity of *Gracillaria edulis* extracts can be attributed to the presence of a diverse array of bioactive compounds with complimentary mechanisms of action, making them promising candidates for therapeutic and nutritional applications (Sunil Kumar *et al*., 2014). *Gracillaria edulis* extracts contain various polyphenolic compounds, including phenolic acids, flavonoids, and tannins, which are well-known for their antioxidant properties. These polyphenols act as potent scavengers of free radicals, effectively neutralizing oxidative stress and protecting cells from damage (Yildrim and Mavi, 2000).

Table: 1. Total Anti-oxidant Potential of Different Sea weed Ethanolic Extracts

Concentration (µg/mL)	*Ulva lactuca*	*Sargassum wightii*	*Gracillaria edulis*
20	0.80 ± 0.001	1.53 ± 0.003	1.69 ± 0.006
40	0.86 ± 0.002	1.62 ± 0.003	1.73 ± 0.008
60	0.89 ± 0.002	1.7 ± 0.003	1.81 ± 0.005
80	0.93 ± 0.002	1.74 ± 0.002	1.86 ± 0.066
100	0.96 ± 0.001	1.78 ± 0.002	1.9 ± 0.005
One-Way ANOVA showed insignificant of activity of p-value 1.582 > 0.05 α-value			

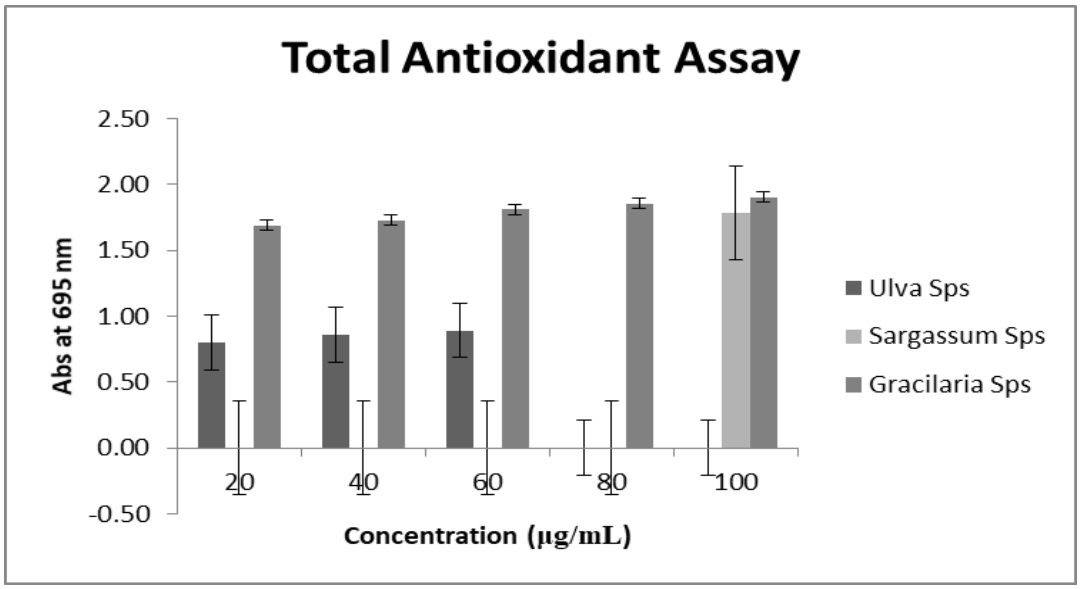

Figure: 1. Total Anti-oxidant Potential of Different Sea weed Ethanolic Extracts

The antioxidant activity of Gracillaria edulis extracts may also be attributed to the synergistic

interactions between various bioactive compounds present in the seaweed. The combined action of polysaccharides, polyphenols, pigments, and other antioxidants may result in enhanced antioxidant potency, making *Gracillaria edulis* extracts highly effective in combating oxidative stress (Sardessai *et al*., 2014).In the reducing power assay, substances with reduction potential interact with potassium ferricyanide (Fe^3+) to generate potassium ferrocyanide (Fe^2+), which subsequently reacts with ferric chloride to form a ferric ferrous complex exhibiting maximal absorption at 700 nm. Notably, the ethanolic extract derived from *Gracillaria edulis* demonstrated a notable and concentration-dependent increase in reducing power. These findings underscore the remarkable antioxidant potential of the extract (Halliwell, 1999).

Antioxidants play a crucial role in preventing oxidative damage caused by reactive oxygen species (ROS) in living organisms. Oxidative stress resulting from an imbalance between ROS production and the body's antioxidant defense mechanisms is implicated in the pathogenesis of various chronic diseases including cancer, cardiovascular diseases, diabetes, and neurodegenerative disorders. Therefore, the search for natural antioxidants from marine sources has intensified in recent years (Singh *et al*., 2014).

The initiation of defense mechanisms against reactive oxygen species (ROS) marks a pivotal response in biological systems. A wide array of enzymes, including superoxide dismutase (SOD), glutathione, catalase, as well as non-enzymatic antioxidants such as flavonoids, α-tocopherol, and ascorbic acid, play crucial roles in combating ROS-induced damage (Sunilkumar *et al*., 2014). The significance of these antioxidants varies depending on factors such as the type of ROS generated, the duration of exposure, and the specific tissue or organ targeted for damage (Gulcin and Oktay, 2002).

Summary and Conclusion:

In conclusion, the findings of this study highlight the differential antioxidant activity present in extracts derived from *Ulva lactuca, Sargassum wightii and Gracillaria edulis* seaweed species. *Gracillaria edulis* extracts demonstrated superior antioxidant activity compared to *Sargassum wightii* and *Ulva lactuca* extracts, suggesting their potential as valuable sources of natural antioxidants. These results contribute to the growing body of evidence supporting the utilization of seaweeds as functional ingredients in the development of antioxidant-rich products for various applications in the food, pharmaceutical, and cosmetic industries. Further research into the specific bioactive compounds responsible for the observed antioxidant activity in *Gracillaria edulis* extracts may provide valuable insights for future therapeutic and nutritional interventions.

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