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Exploring the Antioxidant Potential of Crude Extracts from Turbinaria sp. Seaweed: Towards Sustainable Biomedical Applications

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Investigate the antioxidant potential of Turbinaria sp. seaweed extracts for sustainable biomedical applications. Evaluate antioxidant activity, characterize bioactive compounds, and develop natural antioxidants for pharmaceutical or nutraceutical use.The study focused on the phytochemical screening and antioxidant activity evaluation of Turbinaria sp. seaweed collected from the Tuticorin coastline. The seaweed was processed into a powdered form and extracted using 70% methanol. Phytochemical screening revealed the presence of saponins, phenolic compounds, tannins, alkaloids, steroids, flavonoids, and glycosides. The antioxidant potential was assessed using DPPH assay, nitrous oxide scavenging activity, and hydrogen peroxide scavenging activity. The findings suggest that Turbinaria sp. has promising antioxidant properties, which could be beneficial for various applications.Therefore, our current research focused on assessing the antioxidant properties of an extract from the Turbinaria sp. seaweed at different concentrations (25 - 100µg/ml). The results of seaweed methanolic crude extracts revealed significant DPPH, Nitric oxide scavenging activity & H2O2 activity which prophesied as 51.8%, 55.4% and 62.2% respectively. The findings suggest that the raw extract from Turbinariaseaweed possesses unique antioxidant properties, warranting further investigation to enhance the characterization of its pharmacologically active marine natural compounds.

Keywords: Seaweed, Turbinaria sp., Methanolic extract, Antioxidant, Natural compounds, Marine drugs.

1. Introduction

Seaweeds, also known as marine macroalgae, are non-vascular aquatic plants that are classified into three taxa: green, brown, and red algae. Seaweeds are playing an important role in the marine environment. They provide essential vitamins, minerals, and energy to animals through the CO2 sequestration process in the marine food chains, as well as shelter and nursery grounds for a variety of marine fauna species (Sravaniet al., 2023). Many bio-functional chemicals found in the seaweed, such as pigments and antioxidants, have the potential to inhibit the diseases that are triggered by reactive oxygen species (ROS) (Sanger et al., 2022). Over the past few years, there has been an increasing fascination in natural marine bioactive compounds possessing antioxidant properties due to their potential health benefits and versatile applications across various industries (Petcuet al., 2023). One particularly promising source of such compounds is Turbinaria sp. seaweed, which is known to contain biologically active substances with antioxidant capabilities (Prakosoet al., 2024). Antioxidants are essential in safeguarding living organisms from oxidative stress by neutralizing harmful free radicals and preventing cellular damage. Given the rising demand for natural antioxidants as functional food additives and pharmaceutical ingredients, investigating the antioxidant properties of crude extracts from Turbinaria sp. seaweed holds significant scientific and commercial importance (Rassaeiet al., 2023). Turbinaria sp. belongs to the Sargassaceae family and encompasses several brown algae species found in marine environments worldwide (Galenzogaet al., 2023). These algae have adapted to various ecological niches, leading to the development of distinct chemical compositions, including antioxidants, as a response to environmental challenges like UV radiation exposure and nutrient fluctuations (Parveenet al., 2023). Consequently, Turbinaria sp. seaweed represents an intriguing resource for researchers interested in harnessing its bioactive compounds, particularly those with antioxidant attributes. This study aims to explore and assess the antioxidant potential of crude extracts obtained from Turbinaria sp. seaweed. Using a range of extraction methods and analytical techniques, our goal is to identify and quantify specific antioxidant compounds present in these extracts. Additionally, we are examined the potential applications of Turbinaria sp. seaweed extracts in the food, cosmetics and pharmaceutical industries, where there is a high demand for natural antioxidants due to their perceived health benefits and consumer preference for natural products. Study the antioxidant effects of Turbinaria sp. seaweed extracts not only expands our knowledge of the medicinal potential of marine-derived chemicals, but also supports sustainable seaweed harvesting and use techniques. The present study has the latent to pave the way for the creation of novel antioxidant-rich products for use in functional foods, nutritional supplements, and cosmetics formulations. Finally, this is beneficial to both human health and the environment.

2. Materials and methods:

Reagents:

Ammonium molybdate, PBS, Ascorbic acid, H_2O_2 solution, DPPH, $Na_2[Fe(CN)_5NO]$, H_2SO_4, were purchased from Sigma Chemical Co. (St. Louis, MO).

Collection and process of sample:

The study was conducted at the Marine Biomedical Research lab & Environmental Toxicology Unit, Saveetha Dental College. Turbinaria sp. seaweed was collected from the Tuticorin coastline. The sample was manually collected; epiphytes and the debris were removed by washing in running tap water and

washed again with distilled water. Finally, the seaweed was dried in Hot air oven at 60°C. After drying, the samples were grinned into a coarse powder using mortar and pestle. A twenty gram of powdered sample was taken and then added 200 ml of 70% methanol in the conical flask as well as the sample were kept in the shaker for 3days.Finally, the extract was filtered using Whatman filter paper. The filter samples were placed in a water bath at below 60ºC until it become crude (Fig.1).

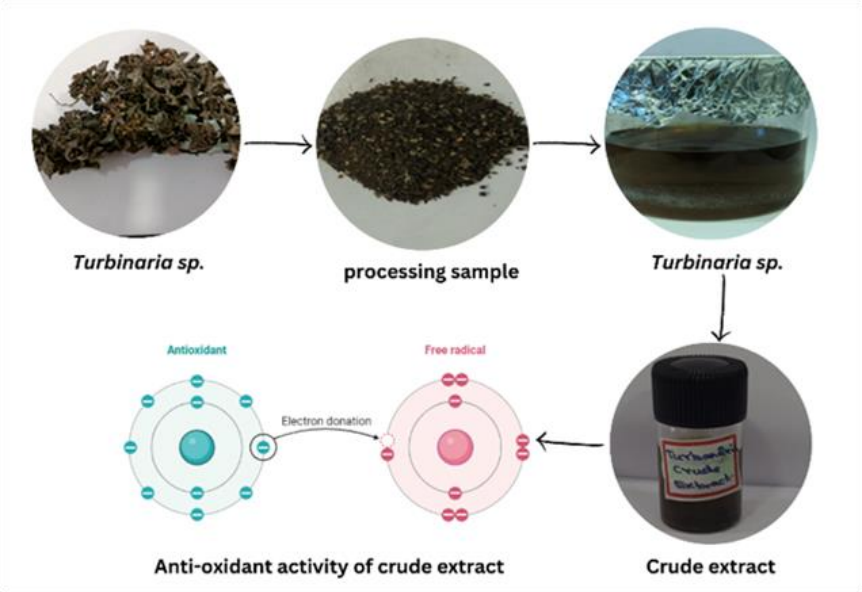

Figure 1 shows the Preparation of crude extract from Turbinaria sp. seaweed

Phytochemical Screening:

The methanol extracts of freshly prepared Turbinaria sp. were examined through a preliminary screening to identify phytochemical components, including saponins, phenolic compounds, tannins, alkaloids, steroids, flavonoids, and glycosides, in accordance with established procedures (Gunasekaranet al., 2019).

Antioxidant activity:

The methanolic extract of Turbinaria sp. was evaluated for its antioxidant potential using the DPPH assay, nitrous oxide scavenging activity, and hydrogen peroxide scavenging activity. This assessment was conducted following the methodology outlined by (Tharaniet al.(2023), with minor adjustments.

3. Results and Discussion

Phytochemical Screening

Preliminary phytochemical analyses of Turbinaria sp. showed the presence of Tannins, saponins, Alkaloids, flavonoids, but there is absence of Steroids and terpenoids (Table 1).Tannins, found in the extract, exhibit antioxidant properties, protecting cells from free radicals that contribute to aging and diseases. Additionally, they can inhibit enzymes, potentially providing anti-inflammatory and anti-cancer effects. Tannins also possess astringent properties, which can aid in wound healing.Saponins, present in the extract, exhibit diverse biological activities, including anti-inflammatory, anti-cancer, and cholesterol-lowering effects. They also act as detergents, foaming agents, and immune system

modulators, with potential applications in the food and pharmaceutical industries.Flavonoids, present in the extract, are antioxidants that protect cells from damage and reduce inflammation. They have potential benefits for heart health and may lower the risk of chronic diseases. Additionally, flavonoids contribute to the extract's color due to their role in plant pigmentation.

Table 1 Phytochemical screening of Turbinaria sp. seaweed extract

Test	Presence	Absence
Tannins	+	
Saponins	+	
Steroid		_
Flavonoids	+	
Terpenoids		_
Alkaloids	+	

Alkaloids, present in the extract, are diverse compounds with various physiological effects. They can act as stimulants, analgesics, or toxins, depending on their type and concentration. Alkaloids are pharmacologically active and have been studied for their potential use in traditional medicine.The absence of steroids in the extract suggests that it may lack typical anti-inflammatory, immunosuppressive, and hormone-regulating properties associated with steroids.The Phyto constituents such as, flavonoids & phenols were found to improve the scavenging of free radicals, as reported by Carissa carandasleaves (Vermaet al., 2015).In addition,Ramarajanet al., 2019quantitatively analysed different solvent extracts of Turbinariaornata and found the presence of steroids, phenols, alkaloids, amino acids, tannins, flavonoids and but no carbohydrates, proteins, terpenoids and saponins. In addition, Deepak et al., 2017reported that the SMME (Sargassummariginatummethanolic extract) has showed the phytochemicals phenol compounds, alkaloids, carbohydrates, glycosides, fats, amino acids, fixed oil, steroids & tannins. (Morsyet al., 2018)has been studied that showed the presence of flavonoids, saponins, alkaloids, phytosteroids, glycosides, carbohydrates, diterpens, phenols, tannins & proteins in the methanol/ chloroform extract of all selected seaweeds (Pterocladiacapillacea, Corallinamediterranea, Corallinaofficinalis, and Ulvalactuca) except tannins, phenols & flavonoids were absent in Corallinamediterranea. However, other phytochemicals present in the extract could still offer medicinal benefits similar to steroids.The absence of terpenoids in the extract suggests it may lack typical anti-inflammatory or antimicrobial effects associated with them. However, other bioactive compounds present could still contribute to its medicinal properties.

Antioxidant Activity

The methanolic extracts from Turbinaria sp. seaweed were assessed for their antioxidant capabilities using various methods, revealing distinct levels of antioxidant activity across different concentrations (25, 50, 75, and 100 µg/ml). The overall antioxidant activity of these extracts was determined by measuring the formation of a green phosphate complex under acidic conditions (Rajeshkumar et al., 2024).

DPPH Assay

The free radical scavenging activity of seaweed (Turbinaria sp.) extracts was calculated by DPPH assay. The results of the DPPH assay on Turbinaria sp. extracts indicate a significant free radical scavenging activity. At a concentration of 100 µg/ml, the extract showed a DPPH scavenging activity of 51.8% (Fig.2). This suggests that Turbinaria sp. extracts have strong antioxidant properties, as they were able to neutralize more than half of the DPPH radicals in the assay.(Novienderiet al., 2023) reported that the assessment of antioxidant activity, the methanolic extract derived from Padina sp. exhibited the peak IC50 value, measuring 362.03 ppm. Conversely, the methanolic extract from Rhodopeltis sp. displayed the lowest IC50 rate at 5.112.30 ppm (Parts per million).

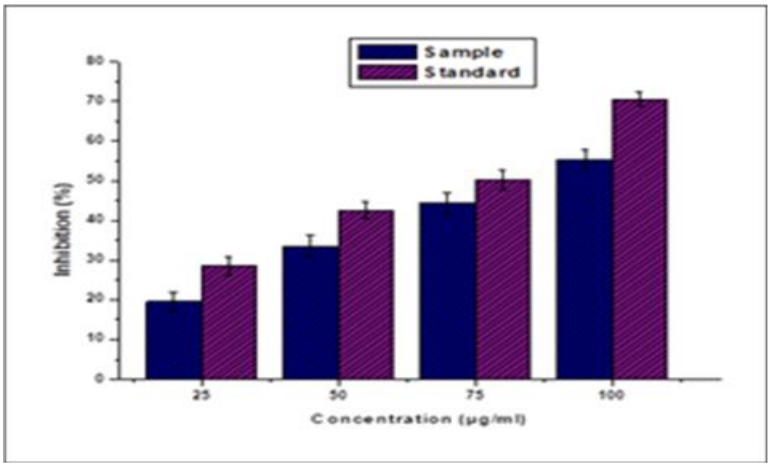

Figure 2 shows the DPPH Scavenging activity of Turbinaria seaweed

As a reference point, ascorbic acid was employed as a positive control in this antioxidant activity test and demonstrated an IC50 value of 5.67 ppm.In 2019 Ahamedet al., found that the extracts from Phomopsis sp. Xylaria sp., Acremonium sp., Cladosporium sp., Aspergillusniger, Chaetomium sp., and Fusarium sp., showed greater antioxidant potential compared to other fungal extracts. Phlorotannin extracted from T. decurrensexhibited the highest DPPH activity, reaching an impressive 92.3% inhibition at a concentration of 100µg/ml. In contrast, previous research reported the lower DPPH inhibition rates, with phlorotannin from S. serratum showing 87.53% inhibition and the DCM (Dichloromethane) fraction of phlorotannin from Cystoseiratrinodis demonstrating 74.66% inhibition (Boiet al., 2017) and (Sathyaet al.,2017). (Kang et al., 2012) observed that dieckol, isolated from E. cava, displayed a robust scavenging effect, even surpassing the performance of the standard ascorbic acid. These results are suggesting that the dieckol present in T. decurrens possesses a notably stronger scavenging activity.These findings highlight the potential of Turbinaria sp. as a source of natural antioxidants, which could have various health benefits, such as reducing oxidative stress and inflammation. Further studies could focus on identifying the specific compounds responsible for this activity and exploring their potential applications in pharmaceuticals or functional foods.

Nitric oxide Assay

The results of the study indicate that the methanolic extract of Turbinaria sp. exhibited a scavenging activity of 55.4% ± 2.6 at a concentration of 100 µg/ml. This activity was compared with the standard antioxidant, ascorbic acid (Fig. 3). The findings suggest that the extract has significant antioxidant properties, comparable to or even exceeding those of ascorbic acid, a well-known antioxidant. The reduction in the release of nitric oxide free radicals may be linked to the ability to directly scavenge nitric oxide free radicals, thereby decreasing the production of nitrite through the oxidation of sodium nitroprusside. The very unstable radical nitric oxide reacts with oxygen molecules to form highly reactive molecules like NO2, N2O4, and N3O4, which can cause a variety of harmful effects. diseases of the body including DNA damage and lipid peroxidation cell damage (Santisoet al., 2012).In this study Patraet al. (2017)reported the UPEO (Undariapinnatifida essential oil) exhibited moderate nitric oxide scavenging potential, with an IC_{50} value of 303.95 mg/ml compared with that of 51.87 mg/ml in the reference compound, a-tocopherol. Recently, Premarathna et al. reported that, the Sargassumilicifolium extracts pointedly stimulated the production of NO in RAW (Cell lines) 264.7 cells at 4 µg/µl concentration when compared to control group (Premarathnaet al., 2023).These results underscore the potential of Turbinaria sp. as a valuable source of natural antioxidants, which could have various health-promoting effects. Further research could explore the specific compounds responsible

for this activity and their potential applications in medicine or functional foods.

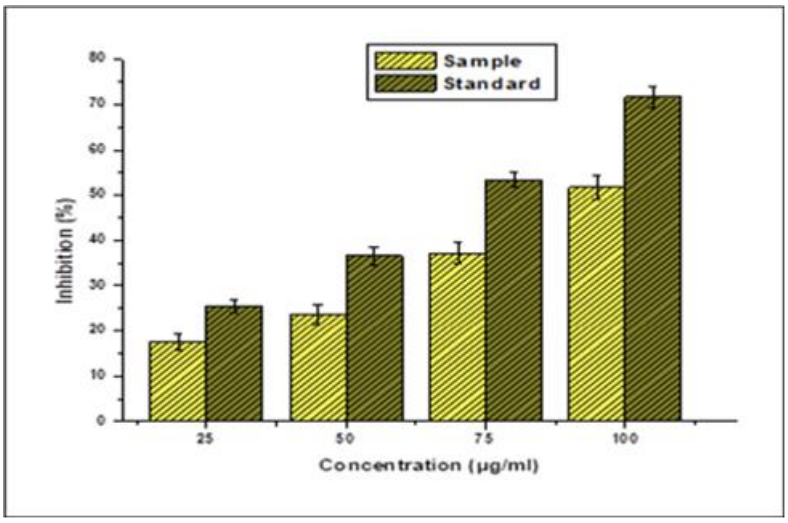

Figure 3 shows the Nitric oxide scavenging ability of Turbinaria seaweed

Hydrogen peroxide assay

The study investigated the scavenging effect of hydroxyl radicals using the Fenton reaction, where the purified extract of Turbinaria sp. seaweed showed significant inhibition of about 62.6% at a concentration of 100 µg/ml (Fig. 4). These results indicate that the extract has potent antioxidant properties against hydroxyl radicals, which are highly reactive and can cause cellular damage. This suggests that Turbinaria sp. seaweed could be a valuable source of antioxidants for potential health benefits. Interestingly, the study also found that the hydrogen peroxide scavenging activity of the extract was higher compared to its DPPH and nitric oxide scavenging activities.Similarly, sulfated polysaccharides from the three seaweeds viz. Sargassumswartzii, Ulvafasciata and Chaetomorphaantennina have been reported to exhibit scavenging activities of 71.2%, 62.7% and 60.3%, respectively at 0.5 mg/ml (Souza et al., 2012).

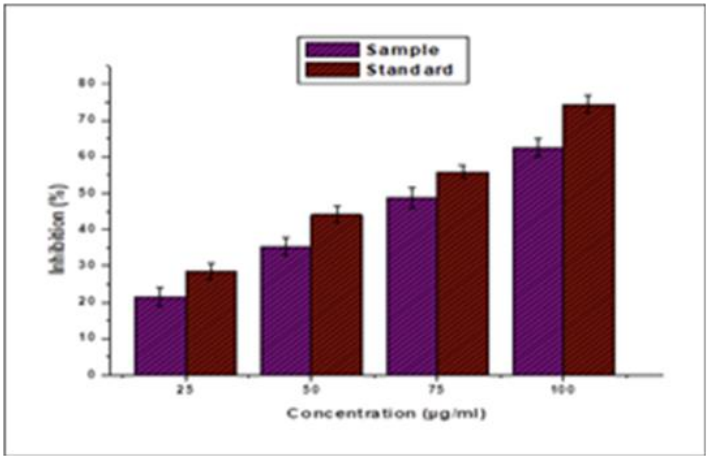

Figure 4 shows the Hydrogen peroxide scavenging activity of seaweed crude extracts

The H_2O_2 radical scavenging abilities of the fractionated polysaccharides were done.FPs isolated from

the three seaweeds was reported by (Venkatesanet al., 2019). When compared to the other concentrations of 25, 50, 100, and 150 g/ml, the fractionated polysaccharides (FPs) extracted from Gracilariafiliforms significantly ($P<0.01$) increased H_2O_2 scavenging activity (82.2 2.35%), followed by FPs from Enteromorphacompressa (80.1 2.35%), and Turbinariaconoides (78.88 1.95%). Ascorbic acid, a common antioxidant, demonstrated 94.22 2.2% H_2O_2 scavenging, completely buffering any potential free radicals. Shenodyet al.,(2019) reported among the seaweeds examined, the total phenolic compound content (TPC) varied from 16.87 ± 3.2 to 474.46 ± 29.3 mg GAE g-1, with the extract of Dictyotadichotoma standing out as significantly higher. The elevated TPC in the D. dichotoma extract directly contributed to its superior antioxidant capacity. This indicates that the extract may have different mechanisms of antioxidant action, targeting different types of free radicals. Overall, these findings highlight the phytochemical richness and antioxidant potential of Turbinaria sp. seaweed, suggesting its possible use in pharmaceuticals or functional foods for health promotion and disease prevention. Further research could explore the specific compounds responsible for these activities and their underlying mechanisms.

4. Conclusion:

The economic significance of the methanol extract derived from the marine seaweed species Turbinaria sp. lies in its demonstrated capacity to scavenge radicals and act as an antioxidant. This is evidenced by its effects on scavenging DPPH, hydrogen peroxide, and nitric oxide. As a result, it can be inferred that this seaweed represents a readily available source of natural antioxidants, potentially leading to health benefits. Furthermore, additional research is required in the marine environment to thoroughly characterize these extracts and explore their potential applications as drugs for human consumption.

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Conflict of interest:

All authors state that, we do not have any conflict of interest.

Data availability:

All data collected during this study are included in this manuscript.

List of abbreviations:

CO_2- Carbon dioxide; DCM- Dichloromethane; DPPH- 2,2-diphenyl-1-picrylhydrazyl; FPs- Fractionated polysaccharides; H_2O_2- Hydrogen peroxide; IC_{50}- Inhibitory concentration; N_2O_4- Dinitrogen tetra oxide; N_3O_4- Dinitroazanide; NO- Nitric Oxide;NO_2- Nitrogen Dioxide; PBS- Phosphate buffer saline; PPM - Parts per million; ROS- Reactive oxygen species; SMME- Sargassummariginatummethanolic extract; Sp.- Species; TPC- Total phenolic compounds; UPEO- Undariapinnatifida essential oil; UV-Ultra violet;

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