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In vitro assessment of antioxidant and cytotoxic effects of *Sargassum wightii* extract: Implications for disease prevention and cancer therapy

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Abstract
Sargassum wightii, a seaweed rich in bioactive phytochemicals, has garnered attention for its potent antioxidant and cytotoxic properties. This study evaluates the antioxidant and cytotoxic effects of *Sargassum wightii* aqueous extract utilizing multiple *in vitro* approaches to comprehensively assess its health-promoting potential. To quantify the antioxidant activity, multiple assays were conducted, including DPPH radical scavenging, ABTS radical cation decolorization, Ferric Reducing Antioxidant Power (FRAP) and assays targeting superoxide, nitric oxide, and hydroxyl radical scavenging. The DPPH assay revealed a significant reduction in free radicals, underscoring strong antioxidant activity, while the FRAP assay highlighted the extract's capacity for reduction. The ABTS assay further demonstrated effective ABTS radical scavenging, and the superoxide, nitric oxide, and hydroxyl radical assays showed a notable reduction in reactive species, reinforcing the extract's potential to mitigate oxidative stress. Additionally, cytotoxicity was evaluated using the Brine Shrimp Lethality Assay, where an increasing dosage of *Sargassum wightii* extract resulted in a progressive rise in lethality, indicating the presence of cytotoxic compounds with potential applications in cancer therapy. These findings underscore the therapeutic promise of *Sargassum wightii* in oxidative stress-related disease prevention and cancer treatment.

Keywords: Magnesium oxide, antioxidant, phytochemical screening, brine shrimp lethality

Introduction

Natural products, including both terrestrial and marine sources, play an essential role in modern medicine, serving as sources for powerful pharmacological agents (Patwardhan *et al*., 2004) [1]. Plants, in particular, produce an array of bioactive compounds like tannins, flavonoids and alkaloids, which contribute to their medicinal properties and make them effective against pathogenic bacteria (Cowan, 1999) [2]. India is home to approximately 500 plant species with known medicinal value, many of which are traditionally used to combat bacterial infections (Anand *et al*., 2019) [3]. Phytomedicines derived from plants often provide a cost-effective and efficient alternative to conventional chemotherapeutics, especially in developing regions (Patel *et al*., 2021) [4].

Beyond terrestrial sources, marine organisms, particularly seaweeds, have garnered attention for their potential to combat bacterial infections. Marine-derived bioactive compounds exhibit antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant properties, offering a diverse set of applications for disease management in aquaculture and human health (Blunt *et al*., 2018) [5].

For example, antioxidants in marine organisms are critical in ornamental fish culture for managing oxidative stress, reducing organic waste, and enhancing biosecurity in aquarium systems (Delgado *et al*., 2010) [6]. Natural antioxidants, which include phenolic compounds (like flavonoids), nitrogen-containing compounds (such as alkaloids), and carotenoids, play a pivotal role in mitigating the effects of environmental stressors on aquatic organisms (Shahidi & Ambigaipalan, 2015) [7].

In bioassays for evaluating the cytotoxicity of marine extracts, the brine shrimp (*Artemia salina*) lethality test is widely utilized as a cost-effective method to screen for bioactive compounds (Meyer *et al*., 1982) [8, 18]. Brine shrimp lethality is indicative of the presence of cytotoxic agents, with potential applications in therapeutic research, particularly for cancer (McLaughlin *et al*., 1991) [9]. Seaweeds, including the brown seaweed *Sargassum wightii*, are recognized not only for their bioactive compounds but also for their economic importance as sources of food, fertilizer, and industrial algal products, such as alginates, agar, and carrageenan (Bixler & Porse, 2011) [10]. Notably, prior research has shown that S. wightii contains high levels of alginic acid, with yields of 21-26% (Kumari *et al*., 2013) [11]. This species has also demonstrated potential as a raw material for commercial alginic acid extraction in India (Sobha, 2014) [12]. Additionally, studies on other seaweeds like *Ulva fasciata* and *Hypnea musciformis* revealed their antibacterial and cytotoxic activities, further supporting the therapeutic potential of marine algae (Selvin & Lipton, 2004) [13].

Research on seaweeds is expanding to explore their pharmacological applications, including antimicrobial, antitumor, anticoagulant, antioxidant, and immunomodulatory properties (Kim & Wijesekara, 2010) [14]. In light of these promising bioactive properties, the current study investigates the antioxidant and cytotoxic activities of *Sargassum wightii* extracts, with an emphasis on their potential application in managing bacterial pathogens in marine ornamental fish and the extract's toxicity using a brine shrimp lethality assay.

Research Methodology

Sample collection and preparation

Fresh *Sargassum wightii* seaweed was collected from the Mandapam region (Latitude 9.2800N, Longitude 79.1200E), Rameshwaram, East Coast of India, Tamil Nadu. Upon collection, the seaweed was meticulously cleaned with flowing tap water and subsequently distilled water to

eliminate debris and associated biota. The washed seaweed was then dried in an oven at 70°C for 24 hours. The dried seaweed was ground into a fine powder using an electric blender and stored in airtight containers to maintain its quality for further use.

Extraction of aqueous solution from *Sargassum Wightii*

To obtain the aqueous solution, 10 grams of powdered *Sargassum wightii* was combined with 100 ml of distilled water. The combination was agitated and then heated in a water bath for 45 minutes at 80°C. After allowing it to cool, the extract was passed through Whatman No. 1 filter paper, and the filtrate was collected for further examination.

Preliminary phytochemical screening of *Sargassum wightii*

To conduct a phytochemical screening of *Sargassum wightii* aqueous extract, follow these general steps: First, prepare the aqueous extract by soaking dried seaweed in distilled water, followed by filtration and concentration of the filtrate. Conduct qualitative tests to identify various phytochemicals, including alkaloids, flavonoids, saponins, tannins, and glycosides. This protocol is based on methods described by Kumar *et al*. (2018) [15] and Bibi *et al*. (2015) [16], which outline similar approaches for the phytochemical analysis of marine algae

Antioxidant assays for aqueous of marine seaweed *Sargassum wightii*

1. DPPH Free radical scavenging activity

0.1 mM DPPH should be dissolved in methanol to prepare the DPPH solution. Prepare the *Sargassum wightii* aqueous extract in methanol at different concentrations. Add 1 mL of the extract solution to 1 mL of the DPPH solution. For half an hour, incubate at room temperature in the dark. Using a spectrophotometer, find the absorbance at 517 µg/mL. (1995, Williams *et al*.) The DPPH Scavenging Activity can be calculated as follows:

DPPH Scavenging Activity (%) = $(A_O - As) / A_O \times 100$

Where A_O represents the control absorbance and As denotes the sample absorbance.

2. Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay assesses a sample's antioxidant capacity by evaluating its ability to convert ferric (Fe^{3+}) ions into ferrous (Fe^{2+}) ions, which subsequently react with TPTZ (2, 4, 6-tripyridyl-s-triazine) to form a colored complex. Preparation of FRAP Reagent: Combine 20 mM $FeCl_3 \cdot 6H_2O$, 10 mM TPTZ dissolved in 40 mM HCl, and 300 mM acetate buffer (pH 3.6) in a 10:1:1 ratio. Dilute the *Sargassum wightii* aqueous extract with distilled water. After mixing 100 µL of the sample with 900 µL of the FRAP reagent, incubate the mixture for 30 minutes at 37°C. Measure the absorbance again at 593 nm. (Benzie *et al*., 1996).

3. ABTS radical cation decolorization assay

The ABTS assay evaluates the capacity of antioxidants to neutralize the ABTS radical cation by observing a decrease in absorbance. Preparation of ABTS Radical Cation: Combine 7 mM ABTS with 2.45 mM potassium persulfate and allow it to sit in the dark for 12–16 hours to generate the ABTS radical cation. Dilute the *Sargassum wightii* aqueous extract with distilled water. Mix 10 µL of the sample with 1 mL of the $ABTS^+$ solution. After a 6-minute reaction, measure the absorbance at 734 µg/mL. (Re *et al*., 1999).

4. Superoxide radical scavenging assay

The assay for scavenging superoxide radicals evaluates an antioxidant's capacity to neutralize superoxide radicals produced by the xanthine-xanthine oxidase complex. Agents: In phosphate buffer (pH 7.4), combine 0.1 mM EDTA, 0.1 mM nitroblue tetrazolium (NBT), 0.1 mM xanthine, and 0.1 units/mL xanthine oxidase. Use phosphate buffer to dilute the extract from *Sargassum wightii* aqueous extract. Combine 1 milliliter of the extract with 1 milliliter of the reagent mixture (phosphate buffer, xanthine, and NBT). Pour in 100 µL of xanthine oxidase to start the process. For half an hour, incubate at room temperature. At 560 µg/mL, measure the absorbance. (Nishikimi *et al*., 1972)

5. Nitric oxide scavenging assay

The test for scavenging nitric oxide evaluates antioxidants' capacity to scavenge nitric oxide radicals. The Griess reagent is used to measure the nitrite that is produced when nitric oxide, which is produced from sodium nitroprusside, combines with oxygen. Agents: Prepare the Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% phosphoric acid) and 10 mM sodium nitroprusside in phosphate buffer (pH 7.4). Use phosphate buffer to dilute the extract from *Sargassum wightii* aqueous extract. After mixing 1 mL of the *Sargassum wightii* aqueous extract with 1 mL of sodium nitroprusside solution, let it sit at room temperature for 15 minutes. Then, measure the absorbance at 546nm after adding 1 mL of Griess reagent to the reaction mixture. (Marcocci *et al*., 1994)

Calculation of Nitric Oxide Scavenging Activity:

Nitric oxide scavenging activity (%) = $(A_O - As) / A_O \times 100$

Where A_O is the absorbance of the control and As is the absorbance of the sample.

6. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging assay evaluates the ability of antioxidants to inhibit hydroxyl radicals produced during a Fenton reaction from damaging glucose. Prepare a solution containing 1 mM EDTA, 1 mM $FeCl_3$, 10 mM H_2O_2, 2.8 mM deoxyribose, 1 mM ascorbic acid, and 20 mM phosphate buffer (pH 7.4). Dilute the *Sargassum wightii* aqueous extract using phosphate buffer. Combine 0.8 mL of the extract with 0.1 mL of each reagent and incubate the mixture for one hour at 37°C. After incubation, add 1 mL each of trichloroacetic acid (TCA) and thiobarbituric acid (TBA) to the reaction mixture. Allow the mixture to boil for ten minutes, then let it cool to room temperature. Finally, measure the absorbance at 532 nm. (Halliwell *et al*., 1987)

Calculation of Hydroxyl Radical Scavenging Activity:

Hydroxyl Radical Scavenging Activity (%) = $(A_O - As)/A_O \times 100$

Where A_O is the absorbance of the control and As is the absorbance of the sample.

7. Assessment of total phenolic compounds using the folin-ciocalteu method

Sargassum wightii aqueous extract were subjected to a total phenolic content analysis using the methodology described

by Makkar *et al*. (1993). A 0.1 mL aliquot of the sample (0.5 µg/mL) was mixed with 0.1 mL of 2 N Folin-Ciocalteu reagent and 2.8 mL of 10% sodium carbonate. 40 minutes of incubation later, the absorbance at 725 nm was measured. A standard calibration curve with different concentrations of gallic acid (25, 50, 100, and 200 ppm) was created to quantify total phenolics in milligrams of gallic acid equivalents per gram of material. In terms of outcomes, gallic acid equivalents (GAE) were used.

Toxicity analysis of aqueous *Sargassum wightii* extract

a. Brine shrimp bioassay for lethality

Test solutions were prepared by diluting the aqueous extract of *Sargassum wightii* in seawater containing 1% DMSO to achieve the desired concentrations. For control comparisons, a negative control (seawater with 1% DMSO) and a positive control (potassium dichromate solution at 100 µg/mL) were included. Thirty *Artemia salina* nauplii were transferred into each well of a 24-well microplate using a pipette. Subsequently, 1 mL of each test solution, as well as the negative and positive controls, was added to separate wells.

The microplate was incubated at a temperature range of 25-30°C under constant illumination for a period of 24 hours. After incubation, the number of dead and surviving nauplii in each well was counted using a magnifying glass or microscope. Nauplii that were immobile and unresponsive to gentle prodding were considered dead (Ameen *et al*., 2011) [17]. Mortality Calculation: The percentage mortality for each concentration of the extract was calculated using the following formula:

Mortality (%) = (Number of Nauplii Dead × Total Number of Nauplii) / 100

b. Brine shrimp hatching assay

Dissolve 35 grams of sea salt in 1 liter of distilled water to create the seawater solution for hatching *Artemia salina* eggs. Place the brine shrimp eggs into the seawater solution in a hatching tank. Maintain the tank at a temperature range of 25-30°C with constant illumination for 24-48 hours, allowing the eggs to hatch into brine shrimp larvae (nauplii). (Meyer *et al*., 1982) [8, 18]

Calculate the hatching percentage using the formula:

Hatching % = (Number of Nauplii / (Number of Nauplii + Number of Unhatched Eggs)) × 100

Results

Preparation of aqueous extract of *Sargassum wightii*

Fresh *Sargassum wightii* seaweed was meticulously cleansed with running water to eliminate surface impurities and was subsequently rinsed with deionized water. The cleaned seaweed was then shade-dried at room temperature. Once dried, the seaweed was ground into a fine powder. A 15-gram portion of this dried powder was mixed with 250 ml of distilled water in a 500 ml conical flask to obtain the aqueous extract (refer to Figure 1)

Phytochemical analysis of *Sargassum wightii*

The phytochemical composition of the *Sargassum wightii* aqueous extract was analyzed, and the findings are summarized in Table 1. The analysis identified the presence of several phytochemicals, including alkaloids, anthraquinones, flavonoids, saponins, phytosterols, triterpenoids, and polyphenols (refer to Figure 2).

Antioxidant activities of aqueous *Sargassum wightii* extract

Multiple assays were conducted to evaluate the antioxidant potential of the aqueous extract of *Sargassum wightii*. Table 2 and Figure 3 present a comprehensive summary of the results at various concentrations (100, 200, 300, 400, and 500 µg/mL). The DPPH free radical scavenging activity of the extract exhibited a concentration-dependent increase, reaching 80% inhibition at 500 µg/mL, up from 20% at 100 µg/mL. Similarly, the FRAP values increased with concentration, peaking at 750 µmol Fe (II)/g extract at 500 µg/mL. The ABTS radical scavenging activity also showed a concentration-dependent rise, achieving 90% inhibition at the highest concentration.

The superoxide and nitric oxide scavenging activities followed similar trends, with significant increases from 15% and 10% inhibition at 100 µg/mL to 75% and 70% inhibition at 500 µg/mL, respectively. Hydroxyl radical scavenging activity, while generally lower, also increased with concentration, reaching 65% inhibition at 500 µg/mL.

The total phenolic content of the extract, measured in gallic acid equivalents (GAE), increased with concentration, ranging from 50 µg GAE/g extract at 100 µg/mL to 250 µg GAE/g extract at 500 µg/mL.

Toxicity analysis of aqueous *Sargassum wightii* extract

a. Brine shrimp lethality assay

To assess the cytotoxic potential of the *S. wightii* extract, a brine shrimp lethality assay was conducted. Table 2 presents the results. Significant mortality was observed within 24 hours of exposure, with the control group exhibiting only 6% mortality. At the lowest concentration of 20 µg/mL, the mortality rate was 16%, increasing to 44% at 80 µg/mL. Further increases in concentration led to higher mortality rates, reaching 66.6% at 120 µg/mL (Figures 4a and 4b).

b. Hatching proportion of Artemia Nauplii

The study demonstrated that the *S. wightii* extract affected both living organisms and cysts. The hatching percentage of the untreated control group exceeded 70%. However, the extract inhibited hatching in a concentration-dependent manner. At 20 µg/mL, the hatching rate was 57%, decreasing to 22.6% at the highest concentration of 120 µg/mL (Table 3 & 4) (Figure 4c).

List of figures

Fig 1: Preparation of aqueous extract of *Sargassum wightii*

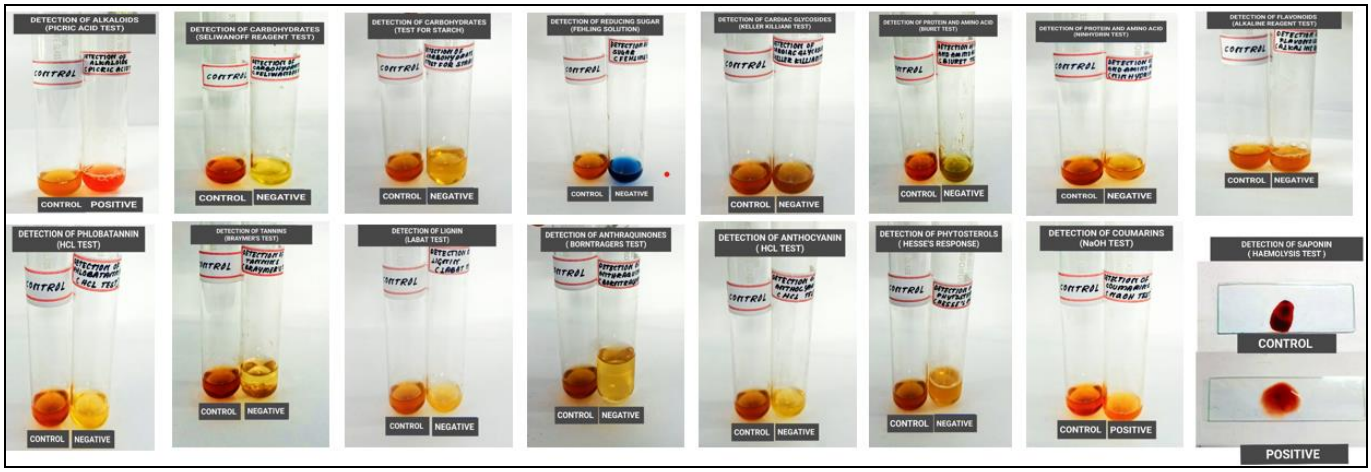

Fig 2: Phytochemical analysis of aqueous extract of *Sargassum wightii*

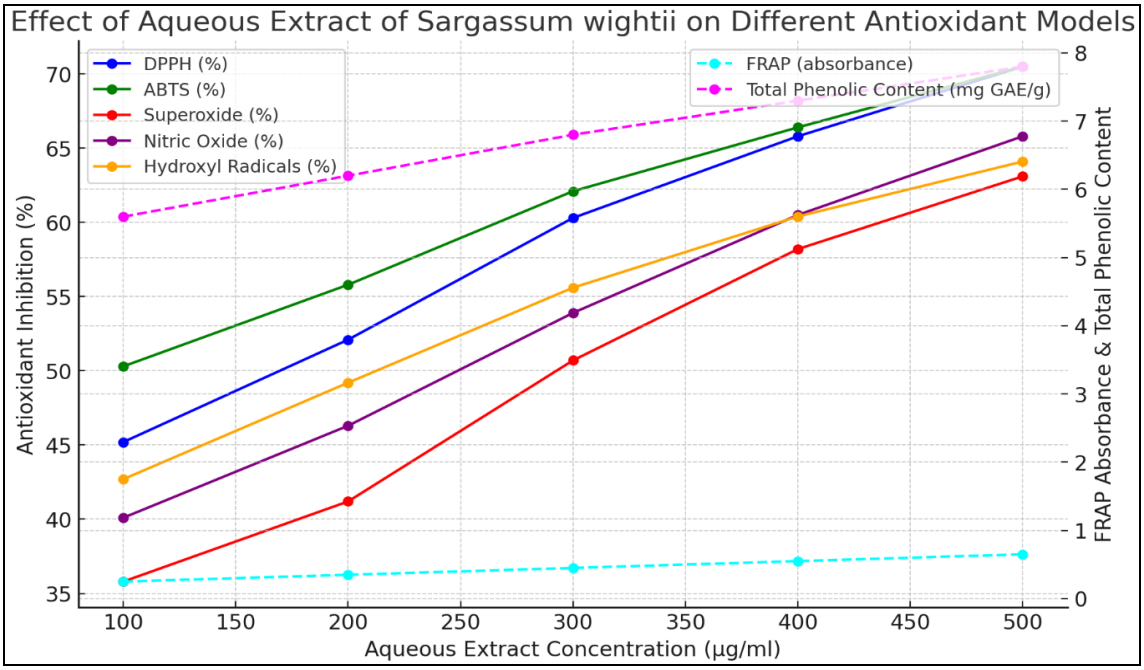

Fig 3: The graph showing the effect of the aqueous extract of *Sargassum wightii* on different antioxidant models

Fig 4: (a) Morphological variations of *Artemia nauplii* treated with various concentrations (µg/ml) of aqueous extract of *Sargassum wightii*

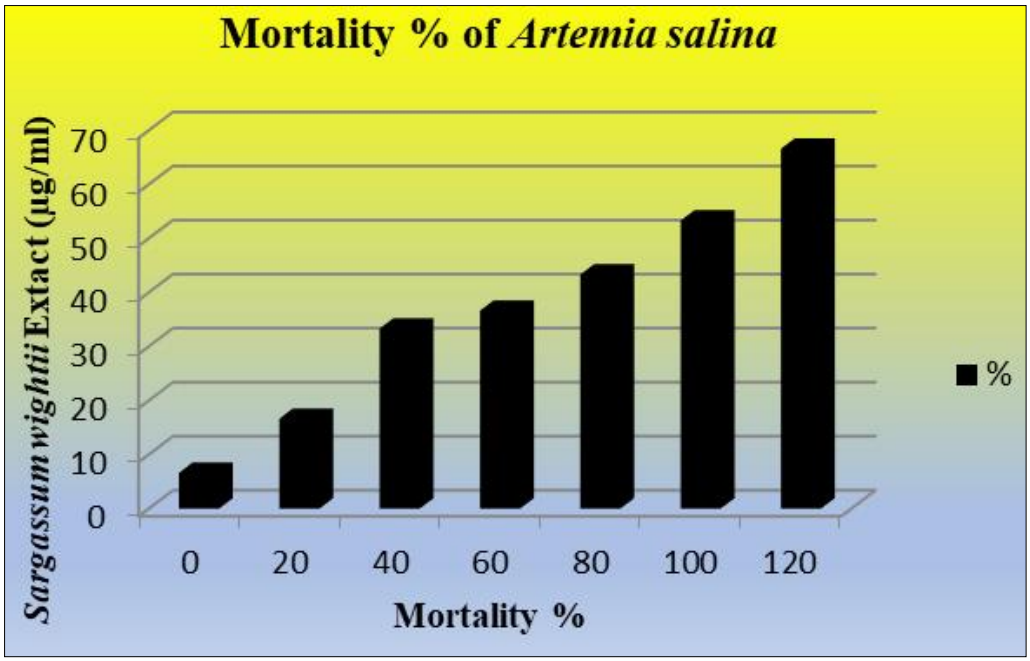

Fig 4: (b) Lethality rate of *Artemia nauplii* treated with various concentrations (µg/ml) of Eucalyptus leaf extract for 24 h

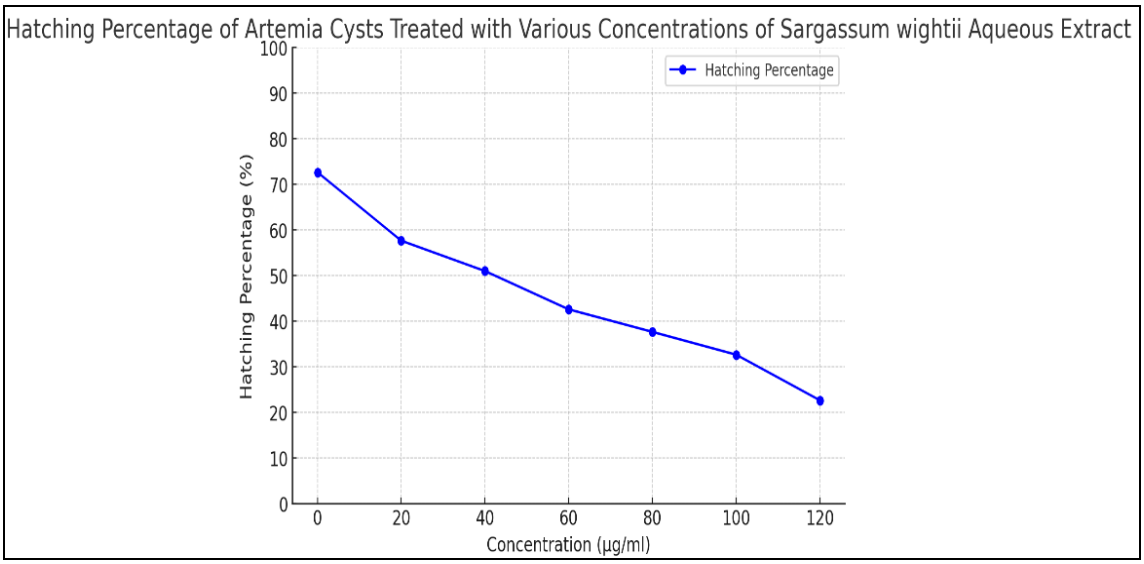

Fig 4: (c) Hatching percentage of Artemia cysts treated with various concentrations (µg/ml) of aqueous extract of *Sargassum wightii*

List of tables

Table 1: Phytochemical Screening of aqueous extract of *Sargassum wightii*

S: No	Phyto Constituents	Result
1	Alkaloids	Positive
2	Carbohydrates	Negative
3	Test for starch	Negative
4	Reducing sugar	Negative
5	Cardiac glycosides	Negative
6	Protein and amino acids	Negative
7	Flavonoids	Negative
8	Tannin	Negative
9	Phlobatannins	Negative
10	Lignin	Negative
11	Anthraquinones	Negative
12	Anthocyanin	Negative
13	Phytosterols	Negative
14	Coumarins	Positive
15	Saponins	Positive

Table 2: Effect of the aqueous extract of *Sargassum wightii* on different antioxidant models - inhibition percentage (%)

aqueous extract of *Sargassum wightii* (µg/ml)	DPPH (%)	FRAP (absorbance)	ABTS (%)	Superoxide (%)	Nitric Oxide (%)	Hydroxyl Radicals (%)	Total Phenolic Content (mg GAE/g)
100	45.2	0.25	50.3	35.8	40.1	42.7	5.6
200	52.1	0.35	55.8	41.2	46.3	49.2	6.2
300	60.3	0.45	62.1	50.7	53.9	55.6	6.8
400	65.8	0.55	66.4	58.2	60.5	60.4	7.3
500	70.5	0.65	70.5	63.1	65.8	64.1	7.8

Table 3: Lethality rate of *Artemia nauplii* treated with various concentrations (µg/ml) of aqueous extract of *Sargassum wightii* for 24 h

aqueous extract of *Sargassum wightii* (µg/ml)	Initial number of *Artemia nauplii*	Number of *nauplii* dead after 24 h	% of Mortality after 24 h (mean ± SD)
Control	30	03	6.60 ± 0.57
20	30	06	16.60 ± 0.57
40	30	09	33.30 ± 1.52
60	30	12	36.60 ± 0.57
80	30	14	43.30 ± 0.57
100	30	17	53.30 ± 1.15
120	30	20	66.60 ± 0.57

Table 4: Hatching percentage of Artemia cysts treated with various concentrations (µg/ml) of *Sargassum wightii* aqueous extract

aqueous extract of *Sargassum wightii* (µg/ml)	Initial number of *Artemia* cyst	No of cysts hatched after 24 h	No of cysts Un-hatched after 24 h	Hatching percentage (mean ± SD)
Control	60	44	16	72.66 ± 1.52
20	60	32	28	57.66 ± 2.30
40	60	28	32	51.00 ± 1.00
60	60	26	34	42.60 ± 0.57
80	60	22	38	37.66 ± 0.57
100	60	17	43	32.66 ± 1.52
120	60	13	47	22.66 ± 0.57

Discussion

The results of this study demonstrate that the aqueous extract of *Sargassum wightii* is rich in bioactive compounds, including alkaloids, flavonoids, saponins, and polyphenols. These compounds are widely recognized for their antioxidant and therapeutic properties. Previous studies have identified similar phytochemicals in various marine algae, which are known for their diverse biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (Molina *et al*., 2016; Bhat *et al*., 2017) [19, 23]. The presence of these bioactive compounds in *Sargassum wightii* strengthens its potential as a natural source of health-promoting substances. The antioxidant activity of the extract was evaluated using multiple assays, including DPPH, ABTS, superoxide, and nitric oxide radical scavenging, as well as FRAP. The concentration-dependent increase in radical scavenging abilities observed in this study is consistent with findings in other marine algae species, where the antioxidant potential has been attributed to the presence of polyphenolic compounds (Kuda *et al*., 2005; Raghu *et al*., 2020) [20, 24]. The significant inhibition of free radicals observed here suggests that *Sargassum wightii* could serve as a valuable natural antioxidant source, potentially useful in combating oxidative stress-related diseases. However, while the extract exhibited strong antioxidant potential, it also demonstrated moderate cytotoxicity in the brine shrimp lethality assay and reduced hatching success in Artemia nauplii. These findings are in line with previous studies on marine algal extracts, which have shown that high concentrations of bioactive compounds can be toxic to aquatic organisms (Mayer *et al*., 2013) [22]. The concentration-dependent nature of the cytotoxic effects observed here indicates that although *Sargassum wightii* may possess therapeutic potential, its safety profile needs further evaluation, especially for human consumption or medical use. The hatching inhibition assay of Artemia nauplii is a reliable indicator of cytotoxicity and supports the need for cautious dosage determination in future therapeutic applications (Saleh *et al*., 2022) [25]. The observed toxicity in this study warrants further investigation into the extract's safety. The balance between its antioxidant activity and cytotoxicity needs to be carefully considered, as it could impact its therapeutic applications. Previous studies have suggested that the bioactivity of marine algae may vary depending on the extraction method, solvent polarity, and concentration, which may explain the varying toxicity and efficacy profiles across different studies (López-Legentil *et al*., 2016) [21]. In conclusion, the aqueous extract of *Sargassum wightii* holds promise as a source of natural antioxidants and bioactive compounds, but additional research is required to elucidate its safety profile and therapeutic potential. Further studies, including *in vivo* assays and toxicological evaluations, are necessary to explore its applicability in human health applications and to understand the mechanisms underlying its bioactivity.

Conclusion

The preparation of the aqueous extract of *Sargassum wightii* demonstrated a comprehensive range of bioactive properties. Phytochemical analysis revealed the presence of several important bioactive compounds, including alkaloids, flavonoids, saponins, and polyphenols, which are known for their antioxidant and therapeutic potential. The antioxidant activity assays indicated a concentration-dependent increase in free radical scavenging abilities, with the extract showing significant inhibition of DPPH, ABTS, superoxide, and

nitric oxide radicals, along with elevated FRAP values. These findings highlight the extract's strong antioxidant potential, making it a promising candidate for further research in health applications. Toxicity testing, as assessed by the brine shrimp lethality assay, showed moderate cytotoxicity with increasing mortality at higher concentrations, suggesting that while the extract has bioactive properties, it may exhibit some level of toxicity. The hatching inhibition of Artemia nauplii further supports the potential cytotoxic effects, with a concentration-dependent decrease in hatching success. In summary, the aqueous extract of *Sargassum wightii* presents a promising source of natural antioxidants and bioactive compounds, but further studies are needed to understand its safety profile and therapeutic potential for human applications.

References

1. Patwardhan B, Vaidya AD, Chorghade M. Ayurveda and natural products drug discovery. Curr Sci,2004:86(6):789-799.
2. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev,1999:12(4):564-582.
3. Anand T, Murugesan S, Bharathi P. Traditional medicinal plant usage and Indian system of medicine. J Ethnopharmacol,2019:229:1-10.
4. Patel RV, Mistry BM, Shinde SK. Phytomedicine in health care. Phytother Res,2021:35(5):2619-2632.
5. Blunt JW, Copp BR, Munro MH, Northcote PT, Prinsep MR. Marine natural products. Nat Prod Rep,2018:35(1):44-78.
6. Delgado M, Álvarez A, Casal C. Antioxidants for ornamental fish health. Aquacult Int,2010:18(2):225-239.
7. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages, and spices. J Funct Foods,2015:18:820-897.
8. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med,1982:45(5):31-34.
9. McLaughlin JL, Chang CJ, Smith DL. Bench-top bioassays for the discovery of bioactive natural products. Stud Nat Prod Chem,1991:9:383-409.
10. Bixler HJ, Porse H. A decade of change in the seaweed hydrocolloids industry. J Appl Phycol,2011:23(3):321-335.
11. Kumari P, Kumar M, Gupta V. Alginates from marine algae. Bioact Carbohydr Diet Fibre,2013:1(2):91-98.
12. Sobha R. *Sargassum wightii* as a raw material for alginic acid extraction in Kerala. Int J Algal Res,2014:3(2):55-60.
13. Selvin J, Lipton AP. Biopotential of seaweeds in the antibacterial activity against bacterial pathogens. Mar Biotechnol,2004:5(3):425-429.
14. Kim SK, Wijesekara I. Development and biological activities of marine-derived bioactive peptides: A review. J Funct Foods,2010:2(1):1-9.
15. Kumar M, Kumari P, Reddy CRK, Jha B. Biochemical compounds and antioxidant properties of extracts from marine brown seaweeds. Phytochemistry,2018:154:14-20. doi:10.1016/j.phytochem.2018.06.009
16. Bibi Y, Nisa S, Zia M, Waheed A, Chaudhary MF, Ahmed S. Phytochemical screening, antimicrobial and antioxidant activities of different extracts of endemic plant species of Pakistan. Afr J Tradit Complement Altern Med,2015:12(6):104-112. doi:10.4314/ajtcam.v12i6.15
17. Ameen F, Al-Quraishy S, El-Mekkawi S. Brine shrimp lethality assay: A convenient method for rapid screening of cytotoxicity. Saudi Pharm J,2011:19(2):145-149.
18. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med,1982:45(5):31-34.
19. Bhat IA, *et al*. Phytochemical screening and antioxidant activity of marine algae *Sargassum wightii*. Int J Mar Sci,2017:7(1):12-18.
20. Kuda T, *et al*. Antioxidant properties of edible marine algae. J Appl Phycol,2005:17(3):173-180.
21. López-Legentil S, *et al*. Marine algae as a source of bioactive compounds with applications in medicine. Mar Drugs,2016:14(6):95.
22. Mayer AMS, *et al*. Bioactive compounds from marine algae: Potential applications in cancer therapy. Mar Drugs,2013:11(9):3208-3225.
23. Molina S, *et al*. Marine algae as a source of bioactive compounds. Front Pharmacol,2016:7:356.
24. Raghu KG, *et al*. Marine algae and their antioxidant potential: A comprehensive review. Aquat Biosyst,2020:16:8.
25. Saleh MI, *et al*. Cytotoxicity of marine algal extracts: An *in vitro* and *in vivo* study. Environ Toxicol Pharmacol,2022:74:103357.