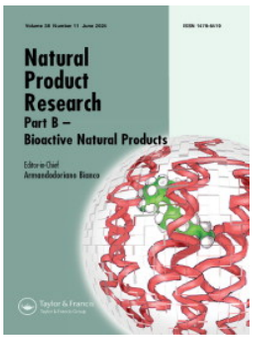

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# Phytochemical profiling, antioxidant potential and cytotoxic activity of *Sargassum prismaticum* extracts: implications for therapeutic applications

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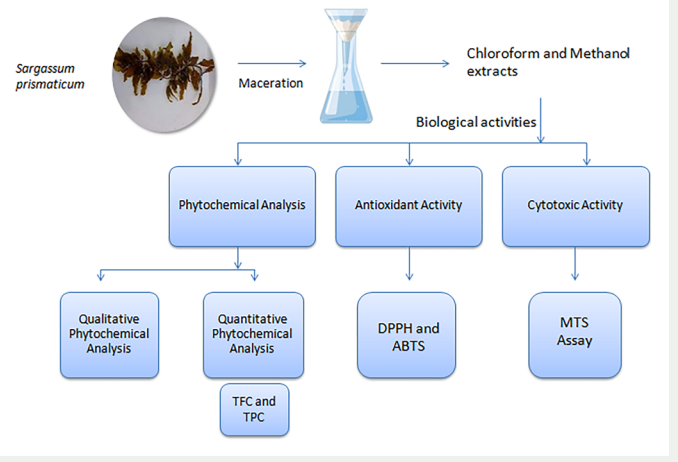
## ABSTRACT

Seaweeds are sources of bioactive compounds with medicinal properties, which make them attractive candidates for natural therapeutic agents. Marine brown algae are known to possess anti-inflammatory, hepatoprotective, anticancer properties, etc. Present study was carried out to identify the phytochemical constituents, antioxidant and cytotoxic activities of *Sargassum prismaticum* in two different solvents viz., chloroform and methanol. Chloroform was found to be the superior solvent for phenol and flavonoid extraction. Antioxidant activity was determined using DPPH and ABTS assays; however, the methanolic extract demonstrated better antioxidant potential. The highest cell cytotoxicity with an $IC_{50}$ value of 7.6±0.02 µg/mL was observed in methanolic extract, while the chloroform extract had an $IC_{50}$ value of 9.6±0.03 µg/mL against U937 cell line. These finding suggest that *Sargassum prismaticum* possesses potent antioxidant and cytotoxic properties, making it a potential candidate for further study as a novel antioxidant drug source.

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## 1. Introduction

Seaweeds are one of the primary producers in their division as lower plants that contain chlorophyll and belong to Kingdom Chromista (Sanjeewa et al. 2017; Dhara and Chakraborty et al. 2021). Their metabolites are regarded as the potent sources of bioactive compounds such as polyphenols, proteins, and lipids, having potential antioxidant, antifungal, antibacterial, and anticancer properties (Akinseye et al. 2017). *Sargassum* (Gulfweed) is brown seaweed which belongs to the order Fucales, and is found to grow in warm waters of tropical and subtropical regions. *Sargassum* has been used to treat diseases like goitre, scrofula, tumour, testicular pain, swelling and edoema (Chakraborty et al. 2009, Chia et al. 2015). The constituents of *Sargassum* vary between different species depending on their growth environments. They have been shown to contain 12–16% proteins, and 1.5– 2.0% lipids (Sethi 2012; Vimala et al. 2015). Assessment of antioxidant and phytochemical constituents of seaweed extracts have been carried out to use them for therapeutic gains (Arunachalam et al. 2008). The presence of antioxidants such as terpenoids, alkaloids, phenols, flavonoids, tannins, pigments, phlorotannin, steroids and glycosides in seaweeds are thought to provide a defence against reactive oxygen species (ROS) resulting from strident environmental conditions (Athukorala et al. 2006). Various studies have reported the antioxidant activity and anticancer effects of these seaweeds (Ale et al. 2011). Other beneficial properties attributed to *Sargassum* include their anti-digestive enzyme activity, anti-inflammatory, hepato-protective and anticancer effects (Atya et al. 2021). cancer-dependent

Past few years have witnessed an increase in cancer-dependent deaths. Cancer is a varied group of disease often distinguished by uncontrolled proliferation of anaplastic cells; most cancer treatments such as chemotherapy and radiotherapy have usual side effects such as chronic pain and peripheral neuropathy etc. (Zandi et al. 2010). The toxic effects of synthetic anticancer drugs have necessitated the search for anticancer compound from natural sources. Crude extracts and compounds isolated from marine algae have been shown to possess anticancer properties. Polysaccharides obtained from brown seaweeds have been shown to possess anticancer activity (Sanjeewa et al. 2017). Phytosterols from microalgae have been targeted for development of chemo preventive drugs for cancer, which induce apoptosis in cancer cells. Oxidative stress is an imbalance between production of reactive oxygen species (ROS), and neutralisation by antioxidants. The consequences of this imbalance is seen on cellular macromolecules and the organisms as a whole (Cuong H.D et al. 2015). Cancer is characterised by three main stages which include initiation, promotion, and progression (Ames and Gold 1992). Oxidative stress has been implicated to promote all the three stages of cancer progression (Klaunig et al. 1998).

## 2. Results and discussion

### *2.1. Antioxidant activity of S. prismaticum*

Antioxidant activity of *S. prismaticum* (*Sargassum prismaticum* V.D Chauhan 1965) is due to the presence of phenolic compounds and flavonoids which are scavengers of ROS. The redox properties of phenolic compounds make them effective reducing

agents and quenchers of ROS and RNS. The antioxidant activities of seaweeds have been attributed to the presence of pigments, phenolic compounds, vitamins, polysaccharides, proteins, etc. (Begum et al. 2021). Among the various algae, brown algae possess highest antioxidant activity (Vladkova et al. 2022).

### 2.2. DPPH and ABTS radical scavenging assays

DPPH and ABTS assays are commonly used to assess the antioxidant potential *in vitro*. DPPH assay indicated that *Sargassum sp*. extracts had an $IC_{50}$ values of 90.5 ± 0.02 and 104.5 ± 0.02 (µg/mL) in methanol and chloroform respectively. Chloroform showed the highest percentage of inhibition at a concentration of 60 µg/mL while the inhibition showed by methanolic extract was 50% at a concentration of 50 µg/mL (Figure S1). The methanolic and chloroform extracts showed $IC_{50}$ values of 54 ± 0.01 and 68 ± 0.02 respectively (Table S3) by ABTS assay. This indicated that methanolic extract had better antioxidant activity when compared to chloroform extract. (Table S3). Results showed that the DPPH radical scavenging activity increased in a concentration-dependent manner at 10–60 µg/mL with 10–60% inhibition of DPPH (Figure S1). The antioxidant activity as determined by ABTS inhibition assay was found to be 54 ± 0.01 and 68 ± 0.02 µg/mL by methanol and chloroform extracts respectively (Table S3). All the concentrations of methanol and chloroform extracts showed similar inhibition of having approximately same percent of inhibition (Figure S2).

### 2.3. Cytotoxicity assay

Brown seaweeds are known to synthesise molecules with pharmaceutical potential to counteract environmental stress. *Sargassum prismaticum* was evaluated for its cytotoxic potential at different concentrations by MTS assay. Results of MTS assay revealed that extracts of *Sargassum prismaticum* were able to hinder the multiplication of the cancer cells. Cell viability was measured based on the absorbance due to reduction of MTS solution by viable cells. U937 cells were treated for 24 h with both the extracts at varying concentrations which showed a significant decrease in cell viability. This was accompanied by alterations in cell morphology after treatment with the extracts (Figure S4). Methanolic extract showed highest cytotoxicity of 73.93% at the concentration of 50 µg/mL while in chloroform extracts showed cytotoxicity of 68.76% at 50 µg/mL concentration. $IC_{50}$ value of methanol and chloroform extracts were 7.6 ± 0.02 µg/mL and 9.6 ± 0.03 µg/mL respectively (Table S4). In present study, the reduction in percent cell viability after 24 h of treatment showed potent cytotoxic effects. Microscopic examination of the cells revealed altered morphology with the presence of cell debris (Figure S4). Earlier studies of Vinayak et al. 2011 have shown that methanolic extracts of various species of *Sargassum* do possess antioxidant and metal chelating properties. Our study further confirms *Sargassum prismaticum* to be a potential source of antioxidants which could be exploited for therapeutic purposes.

Seaweeds are rich sources of bioactive compounds which have medicinal properties making them attractive natural sources of therapeutic compounds. Marine algae inhabit harsh conditions which promote the formation of oxidising agents and secondary metabolites (Dang et al. 2018, Gupta and Abu-Ghannam 2011). These

metabolites, also known as biogenic compounds include alcohols, halogenated compounds, terpenoids, aldehydes, possess anti-algal, anti-bacterial, and anti-fungal properties. (Hakim and Patel 2020). The brown algae are source of two polysaccharides viz., fucoidans and alginate, which have anti-inflammatory anti-cancer, anti-proliferative and anti-viral properties (Kolanjinathan et al. 2014).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced during redox reactions in the organisms. ROS and RNS are required for cellular functions like immune responses, signalling but their excess production causes oxidative stress. ROS and RNS are also produced from external sources like medications, radiations, pollutions leading to generation of oxidative stress (Kim et al. 2014). The human body neutralises the excess oxidative stress by various enzymatic and non-enzymatic antioxidants. However, when the human body is overwhelmed by the free radicals, there is development of degenerative diseases like cataract, cancer, autoimmune disorders, cardiovascular, rheumatoid arthritis, ageing, and neurodegenerative diseases (Perumal et al. 2018). The development of cancer in humans involves molecular and cellular alterations which are mediated by various endogenous and exogenous stimuli. Oxidative DNA damage is a well-known cause for cancer development (Valko et al. 2004). Chemotherapy is commonly used treatment for cancer which generates high oxidative stress in the cells and uses it as a strategy to kill the cancer cells. The therapeutic antioxidants restore the natural antioxidants in the body depleted during chemotherapy. Therefore, they help to increase the survival in patients who have undergone chemotherapy (Singh et al. 2018; Moni et al. 2019). Antioxidants have been shown to be beneficial when combined with chemotherapy (Singh et al. 2018).

Extraction is the first step to obtain bioactive compounds from any organism. Organic solvents ethanol, methanol, acetone, ethanol and their aqueous mixtures have been used for extraction phenols from seaweeds (Koivikko et al. 2005). *S. prismaticum* was screened for the presence of phytochemicals in chloroform and methanol. In this study methanol and chloroform were used as extraction solvents because they have several advantages over water as an extraction solvent. Methanol inhibits the growth of bacteria, mould, non-toxic at low concentrations and less heat is required for concentration of the extracts (Abubakar and Haque 2020). Chloroform has been used for the extraction of fats, oil, flavonoids, etc due to advantages like solubility in solubility in alcohol, sweet smell, easy bioavailability and metabolism in the biological system (Abubakar and Haque 2020). The extracts showed the presence of carbohydrate, protein, alkaloids, flavonoids; saponin and steroids in methanol and chloroform extract (Table S1). Similar results have been reported in the methanolic extracts of brown seaweeds by Sobuj et al. 2021. The extracts were also analysed for the presence of flavonoids and phenols quantitatively. The total phenolic content of *S. prismaticum* was found to be higher in chloroform extract in comparison to methanolic extract (Table S2). There was however no significant difference in the total flavonoid content between the methanolic and chloroform extracts. In the present study chloroform was a better solvent for extraction of phenols and flavonoids. Brown algae have been reported to contain polysaccharides like laminarin, alginic acid fucoidan, and sargassan which are responsible for their anti-cancer activity (Kang et al. 2017). Phenolics comprise of one of the major group of phytochemicals which impart the antioxidant activity to plants or their products (Okpuzor et al. 2009). Flavonoids, on the other hand are the largest group of phenolic

compounds, which are found to occur in free state and as glycosides. Their biological activities including inhibition of mitochondrial adhesion, antimicrobial, antiulcer, antimicrobial, antiulcer, antimicrobial, antiarthritic, protein kinase inhibition, antiangiogenic, anticancer activity, etc. (Bhat et al. 2005).

## 4. Conclusion

*Sargassum prismaticum* could be a potential candidate for further studies as well as a new source of antioxidant drugs.

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## Author's contribution

All authors listed have made a substantial, direct and intellectual contribution to the work.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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