



# Antioxidant and anti-inflammatory activities of methanol and aqueous extracts of *Sargassum wightii*

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## ABSTRACT

**Introduction:** Antioxidants of natural sources for the treatment of many ailments have taken priority since the last decades. Recently, researches have been focused on marine algae as they are the largest reservoir of bioactive compounds. Hence, the objective of this study was to explore the in-vitro antioxidant and anti-inflammatory activities of methanolic and aqueous extracts of *Sargassum wightii*.

**Methods:** The total phenolics, flavonoids, and ascorbic acid (AA) contents were evaluated in forms of gallic acid equivalent (GAE), rutin equivalent (RUE), and AA equivalent, respectively. The aqueous and methanolic extracts were isolated. The antioxidant activities were explored using 2,2-diphenyl-1-picrylhydrazil (DPPH), superoxide dismutase (SOD), hydroxyl radical scavenging, and ferric reducing power assays. The *in vitro* anti-inflammatory activity was assayed using nitric oxide radical scavenging, inhibition of protein denaturation, and antiproteinase activities.

**Results:** We observed significant changes in DPPH scavenging activity with both methanolic *Sargassum* extract (MSE) and aqueous *Sargassum* extract (ASE) [IC<sub>50</sub>: 511.15 µg/mL and 927.05 µg/mL, respectively]. Methanolic extract showed a greater SOD scavenging activity [IC<sub>50</sub>: 369.56 µg/mL] and hydroxyl radical scavenging potential [IC<sub>50</sub>: 668.93 µg/mL] than that of ASE [SOD, IC<sub>50</sub>: 923.94 µg/mL; hydroxyl ion, IC<sub>50</sub>: 953.57 µg/mL]. In the Ferric reducing antioxidant power assay, MSE and ASE exhibited absorbance of 0.93 ± 0.12 and 0.59 ± 0.08, respectively, at 1200 µg/mL each. Both methanol and ASEs showed NO<sup>-</sup> scavenging activity having IC<sub>50</sub> in order, AA (96.46 µg/mL) < MSE (963.50 µg/mL) < ASE (1974.88 µg/mL). However, the protein denaturation inhibition and antiproteinase activity of both these extracts at 1000 µg/mL were similar.

**Conclusion:** *Sargassum wightii* has promising antioxidant and anti-inflammatory activities and could be a potential candidate for drug development targeting oxidative stress-mediated inflammatory diseases.

### Implication for health policy/practice/research/medical education:

This study evaluated the antioxidant and anti-inflammatory activities of methanolic and aqueous extracts of *Sargassum wightii* using in vitro tests. *Sargassum wightii* is a brown seaweed highly nutritious, a rich source of secondary bioactive components, and used as a dietary supplement with promising therapeutic potential to manage oxidative stress-induced neurological disorders, cognitive ability, and neuroinflammatory diseases.

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## Introduction

The oxidative stress due to reactive oxygen species (ROS) and reactive nitrogen species are considered as the harmful products of usual metabolic processes in living organisms. The most important radical derivatives of ROS include superoxide anion (O<sub>2</sub><sup>-</sup>), hydroxyl radical

(OH<sup>-</sup>), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Hydrogen peroxide released from many biochemical reactions in the body can generate hydroxyl radical which is the most dangerous inciting agent among all disorders (1). Enormous evidence supports the role of these free radical-induced oxidative damage to the cell components in the

pathogenesis of a wide range of diseases such as cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders (2). Additionally, the involvement of oxidative stress-induced protein denaturation in inflammatory processes with these diseases provides insight for the development of antioxidants targeting oxidative stress-mediated inflammation and subsequent cell damage.

Over the past decade, considerable attention has been centered on researches related to marine macroalgae as they are the largest reservoir of structurally diverse, bioactive compounds and secondary metabolites (3-6). Though the anti-inflammatory, anticoagulant, antimicrobial, anticancer, antiviral, antioxidant, hypoglycaemic, antiulcer, neuroprotective, and hepatoprotective properties of seaweeds are well documented, the phytochemicals attributed to these properties are yet to be explored (7-12). Among marine algae, the brown seaweeds collected from different coasts across the globe have been widely studied. However, evidence on the pharmacological activities of *Sargassum wightii* (brown seaweed), especially indigenous to Indian coast is scanty. In our previous study, the phytoconstituents of methanolic and aqueous extracts of *S. wightii* were analysed and found to contain mostly the phenolic and flavonoid constituents (13). The antioxidant activity of plants attributed to their phenolic constituents addressed this research to investigate the antioxidant and anti-inflammatory activities of the methanolic and aqueous extracts of *S. wightii* using *in vitro* test models with hope for its food and nutraceutical applications.

## Materials and Methods

### Plant material and extraction process

The plant material was a gift sample from Microbiotech limited (Manufacturers of natural agricultural inputs) New Gundlav, Valsad, Gujarat, India, date 04.01.2017. The dried samples were coarsely ground to a fine powder using an electrical blender before extraction. To prepare a methanolic extract of *Sargassum*, 40 g of the powder sample was extracted with 400 mL of methanol using Soxhlet's apparatus for 72 hours. Using distilled water as a solvent, the same procedure was followed for preparing the aqueous extract. The extracts obtained were dried in an evaporator and stored at -20°C for further use (12). Different concentrations of drug solutions were freshly prepared on the day of the experiment using respective solvents.

### Chemicals

2,2-Diphenyl-1-picrylhydrazil (DPPH), diclofenac sodium, potassium ferricyanide, and trichloroacetic acid (TCA) were purchased from Sigma Aldrich USA. Folin-Ciocalteu reagent, potassium superoxide, methanol, ferric chloride, phosphate buffer, Tris buffer, trypsin, bovine serum albumin, and all other chemicals were obtained from Merck Company. All chemicals were of analytical grade.

### Phytochemical screening of plant extract

The methanolic and aqueous extracts of *S. wightii* were subjected to phytochemical screening using standard methods (14). They revealed the presence of polyphenols, terpenoids, tannins, flavonoids, polysaccharides like glycolipids, etc.

### Determination of total phenolic contents

Total phenolic contents of the crude methanolic and aqueous extracts of *S. wightii* were measured by using Folin-Ciocalteu's method as described by Taga et al. To 100 µL aliquot of the sample, 2 mL of 2% Na<sub>2</sub>CO<sub>3</sub> was added, mixed, and allowed to stand for 2 min at room temperature. After incubation, 100 µL of 50% Folin-Ciocalteu's phenol reagent was added, mixed thoroughly, and allowed to stand for 30 minutes at room temperature in dark. The absorbance of all samples was measured at 720 nm using a spectrophotometer (Shimadzu UV-1800), and total phenolic content was expressed as gallic acid equivalent per gram (GAE/g) of dry extracts considering gallic acid as standard (15).

### Determination of total flavonoid contents

Determination of flavonoids content was done by the Aluminum Chloride Colorimetric method (16). Rutin in the range of 20-200 mg/L was used for the calibration curve. Aluminum chloride (0.5 ml of 2%) was mixed with an equal volume of the extracts of *S. wightii*, incubated for 1 hour at room temperature, and the absorbance of the mixture was measured at 415 nm using a UV/Visible Spectrophotometer. All estimations were carried out in triplicates and their mean values were expressed as mg of Rutin equivalents per gram dry weight (RUE/g) (16).

### Determination of ascorbic acid

The method of Sadashivam et al was followed for the estimation of vitamin C content using 2,6-dichlorophenol indole phenol as a blue dye indicator for titration. The values were expressed as mg of ascorbic acid equivalents per gram (AAE/g) of plant extracts (17).

### Total antioxidant activity

The total antioxidant activity of the plant extracts was determined using the phosphomolybdate method as described by Prieto et al. The mixture was incubated at 90°C for 30 minutes and the absorbance was measured spectrophotometrically at 695 nm against a blank. The total antioxidant activity was expressed as mg of AA equivalents per gram of plant extract on a dry weight basis (18).

### Determination of antioxidant activity

#### DPPH radical scavenging activity

The method of Yen and Chen was followed for the determination of the DPPH radical scavenging activity of *S. wightii* extracts. Different concentrations (50-1000

µg/mL) of the test sample (2.0 mL) was added to 2.0 mL of 0.16mM DPPH methanolic solution. The mixture was vortexed for 1 minute and allowed to stand for 30 minutes at room temperature in dark. The absorbance of all samples was measured spectrophotometrically at 517 nm. The scavenging activity was calculated using the following equation (19):

$$\text{Scavenging effect(\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where  $A_{\text{control}}$  means absorbance of the control (DPPH solution without sample);  $A_{\text{sample}}$  means absorbance of the sample (test sample + DPPH solution)

AA was used as the reference standard. The results were expressed as  $IC_{50}$  as obtained from the linear regression and that means the concentration at which DPPH is quenched by 50%. A lower  $IC_{50}$  value indicates a higher antioxidant activity.

#### Hydroxyl radical scavenging activity

Hydroxy radical scavenging activity was measured using the Fenton reaction (20). For this assay, different concentrations of the extracts were mixed with the Fenton reaction mixture containing 3mM deoxyribose, 0.1mM ferric chloride, 0.1 mM EDTA, 0.1mM AA, and 2mM  $H_2O_2$  in 20mM phosphate buffer pH 7.4. The reaction mixture was incubated at 37°C for 30 minutes. Half ml of 5% TCA and 0.5 mL of 1% thiobarbituric acid were added to the above mixture and placed in a boiling water bath for 30 minutes and cooled. The absorbance was noted spectrophotometrically at 532 nm against blank. The percentage of scavenging activity was calculated as per the above formula (20).

#### Superoxide scavenging activity

This test was performed using the alkaline dimethyl sulfoxide (DMSO) method (21). DMSO and solid potassium superoxide were allowed to stand for 24 hours. Freshly filtered solution (200 µL) was added to 2.8 mL of nitroblue tetrazolium (56 µmol), ethylene diamine tetraacetic acid (EDTA) and potassium phosphate buffer (10 mmol, pH 7.4). Different concentrations of the test solutions were added and the absorbance was recorded at 560 nm against blank reagent (pure DMSO) (21).

#### Ferric reducing antioxidant power (FRAP) assay

The method of Oyaziu was followed for measuring reducing power. Different concentrations of the plant extracts (1.0 mL) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferric cyanide (1%). The mixture was incubated at 50°C in a water bath for 20 minutes. Then, 2.5 mL of TCA (10%) was added and centrifuged for 10 minutes at a rate of 650 rpm. To 2.5 mL of supernatant layer solution, 2.5 mL of distilled water and 0.5 ml of ferric chloride (0.1%) were added. The absorbance was measured at 700 nm (22).

#### *In vitro* anti-inflammatory activity

##### Nitric oxide scavenging activity

In this test, different concentrations of test solutions were dissolved in methanol and incubated at 25°C for 30 minutes. Then, 1.5 mL of the test solution was added with 1.5 mL of Griess reagent. (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance was measured at 546 nm against the control. Sodium nitroprusside (5 mmol) was mixed with the control solution only (23).

##### Inhibition of protein denaturation

To evaluate the anti-inflammatory activity of the extracts, a previously established method was followed (24,25). One ml of a test sample of different concentrations (100, 200, 500, and 1000 µg/mL) was homogenised with 1 mL of the aqueous solution of 5% bovine serum albumin and incubated at 27°C for 15 minutes. The mixture of distilled water and bovine serum albumin was served as the control. Denaturation of proteins was done by placing the mixture in a water bath for 10 minutes at 70°C. Then the mixture was allowed to cool at room temperature and the absorbance was taken at 660 nm using a spectrophotometer (SHIMADZU UV-1800). Each test was performed in triplicate and the following formula was used to calculate the percentage inhibition (24,25):

$$\text{Percentage Inhibition (\%)} = [(Abs \text{ of Control} - Abs \text{ of sample}) / Abs \text{ of control}] \times 100.$$

##### Antiproteinase activity

To perform the antiproteinase activity, the modified method of Sakat et al and Oyedepo et al was followed. The reaction mixture containing 2 ml of 0.06 mg trypsin, 1 mL of 20mM Tris HCl buffer (7.4) and 1 mL of a test sample of different concentrations (100-1000 µg/mL) was prepared and incubated at 37°C for 5 minutes, and 1 mL of 0.8% (w/v) casein was added, then the mixture was incubated for another 20 minutes. Two milliliters of 70% perchloric acid was added and the cloudy suspension was centrifuged. The absorbance of the supernatant solution was recorded at 210 nm and the buffer served as blank. The mean value of the three observations was noted. The percentage inhibition of proteinase inhibitory activity was calculated by using the above formula (26,27).

#### Statistical analysis

Descriptive statistics was applied to express the data using GraphPad Prism software. The values were expressed as mean  $\pm$  SEM. Unpaired *t* test were applied to compare the difference between mean of test drugs and standard.

#### Results

Preliminary phytochemical analysis of the methanolic extract of *S. wightii* revealed the presence of phenols, flavonoids, steroids, reducing sugar, tannins, and saponins.

### Total phenolic and flavonoid contents of *Sargassum wightii* extracts

Total phenolic and flavonoid contents of *S. wightii* were quantitatively varied according to solvents extraction processes. The highest total phenolics ( $2.74 \pm 0.17$  mg GAE/g dry wt) and flavonoids ( $2.62 \pm 0.07$  mg RUE/g) dry wt were recorded in the crude methanol extract of *Sargassum wightii*, which were significantly higher than that of aqueous extract ( $P < 0.01$ ). The methanol extract had high yield value during quantitative estimation than that of aqueous extract, due to the extraction of both non-polar and polar active compounds from the crude extract.

### Ascorbic acid content

It was observed that the aqueous extract contained a significantly higher quantum of AA than methanolic extract ( $2.65 \pm 0.13$  mg vs  $1.53 \pm 0.19$  mg) per gram dry weight (Table 1).

### Total antioxidant activity

In this work, a non-significant higher antioxidant activity was exhibited by methanolic extract of *S. wightii* than that of aqueous extract ( $2.1 \pm 0.07$  vs  $1.87 \pm 0.09$  mg of AA equivalents/gram of dry extract) (Table 1).

### DPPH radical scavenging activity

All the samples exhibited a concentration-dependent increase in 2,2-diphenyl-1-picrylhydrazil (DPPH) scavenging activity (Figure 1A).  $IC_{50}$  values were calculated from the regression line. AA showed the strongest DPPH scavenging activity with  $IC_{50}$  value  $15.6205 \mu\text{g/mL}$  whereas, MSE and ASE exhibited high scavenging ability with  $IC_{50}$  values  $511.15 \mu\text{g/mL}$  and  $927.05 \mu\text{g/mL}$ , respectively. At  $1000 \mu\text{g/mL}$  of each sample, the standard AA showed a highly significant inhibition than both extracts of *S. wightii* (AA  $-98.87 \pm 0.61\%$ , MSE  $-77.29 \pm 1.8\%$  and ASE  $56.27 \pm 1.67\%$  inhibition).

### Hydroxy radical scavenging activity

Figure 1B shows the hydroxyl radical scavenging activity of all samples revealing a concentration-dependent increase in scavenging activity. The ascorbic acid at  $1000 \mu\text{g/mL}$  exhibited the highest scavenging activity ( $82.87 \pm 1.54\%$ ) followed by methanolic sargassum extract (MSE) ( $62.06 \pm 2.22\%$ ) and aqueous sargassum extract (ASE) ( $45.63 \pm 2.05\%$ ) at  $1000 \mu\text{g/mL}$  each. The  $IC_{50}$  values

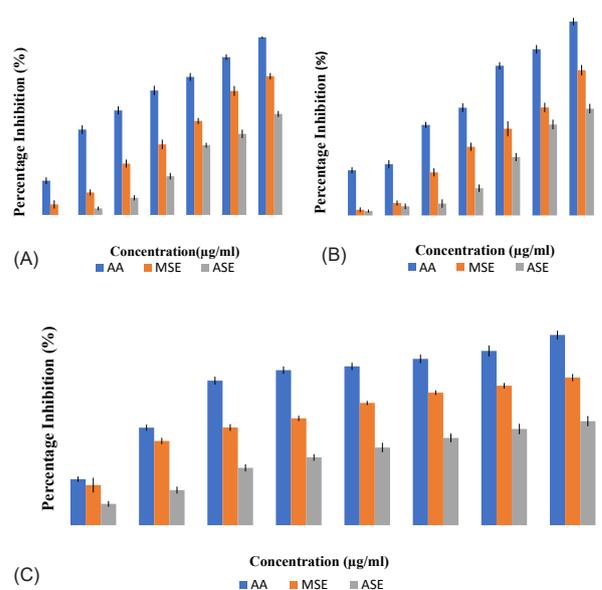
of all samples in descending order  $AA < MSE < ASE$  were  $295.33 < 668.93 < 953.57 \mu\text{g/mL}$ , respectively. No significant difference was observed in scavenging activity between MSE and ASE ( $P > 0.05$ )

### Superoxide dismutase scavenging activity

A  $67.78 \pm 1.88\%$  inhibition of superoxide radical scavenging activity was shown by AA at  $100 \mu\text{g/mL}$ , which was significantly higher than both extracts of *S. wightii* ( $P < 0.001$ ) at corresponding concentrations. A moderate inhibition was observed with methanolic sargassum extract and ASE having  $IC_{50}$  values of  $369.56 \mu\text{g/mL}$  and  $923.941 \mu\text{g/mL}$ , respectively (Figure 1C). The  $IC_{50}$  of AA was much lower ( $45.632 \mu\text{g/mL}$ ) revealing the strongest scavenging ability.

### Ferric reducing antioxidant power

The reducing power of all samples increased with the increasing concentrations of the extracts and the reference standard AA (Figure 2). At  $1200 \mu\text{g/mL}$ , AA showed higher reducing activity ( $3.92 \pm 0.092$ ) than methanolic



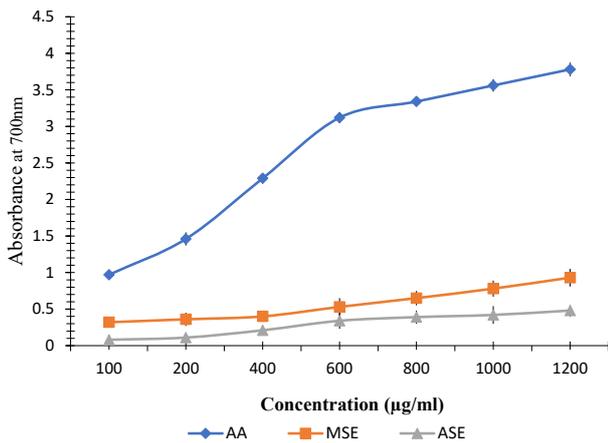
**Figure 1.** Different free radical scavenging activities of *Sargassum wightii* extracts at various concentrations. Ascorbic acid (AA), Methanolic Sargassum Extract (MSE), and Aqueous Sargassum Extract (ASE). A: DPPH scavenging activity, B: Hydroxyl radical scavenging activity, C: Superoxide inhibition.

**Table 1.** Total yield, phenolic, flavonoid, and ascorbic acid contents of *Sargassum wightii* extracts

Extracts	Total yield(%w/w)	Total phenolics (mg GAE/g dry wt)	Total flavonoids (mg RUE/g dry wt)	Total ascorbic acid content (mg AA/g dry wt)	Total antioxidant activity (mg AA/g dry wt)
MSE	5.68	$2.74 \pm 0.17^{**}$	$2.62 \pm 0.07^{**}$	$1.53 \pm 0.19$	$2.17 \pm 0.07$
ASE	2.94	$1.14 \pm 0.35$	$1.21 \pm 0.05$	$2.65 \pm 0.13^{**}$	$1.87 \pm 0.09^{ns}$

MSE, methanolic Sargassum extract; ASE, aqueous Sargassum extract; GAE, gallic acid equivalent.

Values are means of triplicates  $\pm$  SEM. An unpaired 't' test was applied to compare the respective parameters of methanolic and aqueous extracts of *S. wightii*.  $^{**}P < 0.01$  (methanolic vs aqueous);  $^{ns}$  not significant difference.



**Figure 2.** The absorbance values for ascorbic acid, methanol and aqueous *Sargassum* extracts at concentration series in ferric reducing antioxidant power assay. AA, ascorbic acid; MSE, methanolic *Sargassum* extract; ASE, aqueous *Sargassum* extract.

extract followed by aqueous extract ( $0.93 \pm 0.12$  and  $0.59 \pm 0.08$ , respectively).

### In-vitro anti-inflammatory activities

#### Nitric oxide scavenging activity

The nitric oxide (NO) scavenging potential of all samples was again concentration-dependent. There was a  $54.89 \pm 2.4$  % inhibition of activity shown by 100 µg/mL of AA. At the corresponding concentrations, methanolic and aqueous extract of *S. wightii* had  $36.39 \pm 2.05\%$  and  $20.65 \pm 1.19\%$  scavenging activity respectively (Figure 3). The  $IC_{50}$  values in decreasing order were AA ( $96.46$  µg/mL) < MSE ( $963.50$  µg/mL) < ASE ( $1974.88$  µg/mL).

#### Inhibition of protein denaturation

In this study, both methanolic and ASEs inhibited heat-induced protein (albumin) denaturation. Table 2 shows  $47.26 \pm 1.23\%$  and  $41.0 \pm 1.01\%$  inhibition of protein

denaturation for methanolic extract and aqueous extract, respectively at 1000 µg/mL each whereas, diclofenac sodium produced  $92.26 \pm 1.41\%$  inhibition ( $P < 0.001$ ).

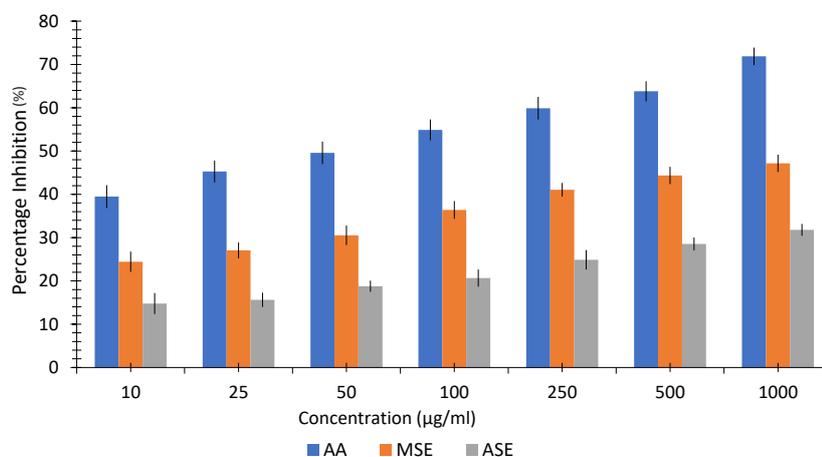
#### Antiproteinase activity

Both MSE and ASE exhibited maximum antiproteinase activity at 1000 µg/mL, which were  $56.35 \pm 1.22\%$  and  $51.14 \pm 1.54\%$ , respectively as shown in Table 2. Diclofenac sodium showed maximum inhibition ( $67.91 \pm 0.84\%$ ) at 1000 µg/mL.

### Discussion

The ROS are generated in large amounts during oxidative stress in the forms of superoxide anion ( $O_2^-$ ), hydroxyl radical (OH), hydrogen peroxide ( $H_2O_2$ ), and NO. These are involved in the pathogenesis of various diseases in human beings. Moreover, oxidative stress also induces certain inflammatory reactions that damage various proteins in tissues. The antioxidant system protects against oxidative damage. Synthetic antioxidants like butylated hydroxyl toluene (BHT) or BHA have found limited use due to their suspected carcinogenic effect (28). Therefore, the natural antioxidants of plant origin have taken the upper hand and have been intensely used to modulate oxidative stress in modern life (29). Among the natural antioxidants, marine seaweeds are now being considered a rich source of antioxidants. Hence, the present work was carried out to screen the potential free radical scavenging and anti-inflammatory activities of *Sargassum wightii* using different *in vitro* test models.

Phenolic compounds have been reported to have several biological activities including antioxidant properties. Earlier reports revealed that the polyphenols like phlorotannins and fucoxanthins present in marine seaweeds help to overcome oxidative stress (30). In the present study, the total phenolic and flavonoid contents in methanolic and aqueous extracts of *S. wightii* were



**Figure 3.** The percentage inhibition of nitric oxide scavenging activities of ascorbic acid and *Sargassum wightii* extracts at different concentrations. AA, ascorbic acid; MSE, methanolic *Sargassum* extract; ASE, aqueous *Sargassum* extract.

**Table 2.** Effect of methanolic and aqueous extracts of *Sargassum wightii* on protein denaturation and antiproteinase activity

Drug	Concentration ( $\mu\text{g}/\text{mL}$ )	% Inhibition of protein denaturation	% Inhibition of proteinase action
Diclofenac sodium	100	49.33 $\pm$ 0.88	40.54 $\pm$ 0.43
	250	58.26 $\pm$ 0.55	50.58 $\pm$ 0.48
	500	74.77 $\pm$ 1.3	58.12 $\pm$ 0.6
	1000	92.26 $\pm$ 1.40	67.91 $\pm$ 0.84
Methanolic <i>S. wightii</i>	100	35.18 $\pm$ 1.48	37.15 $\pm$ 0.7
	250	37.77 $\pm$ 1.03	41.23 $\pm$ 0.74
	500	41.11 $\pm$ 1.10	48.02 $\pm$ 0.88
	1000	47.26 $\pm$ 1.23	56.35 $\pm$ 1.22
Aqueous <i>S. wightii</i>	100	27.47 $\pm$ 0.66	29.63 $\pm$ 0.97
	250	29.06 $\pm$ 0.64	34.03 $\pm$ 0.91
	500	34.5 $\pm$ 0.84	42.02 $\pm$ 0.92
	1000	41.0 $\pm$ 1.01	51.14 $\pm$ 1.54

The percentage values were obtained using various concentrations of the test samples. Readings are expressed as the mean of three readings  $\pm$  SEM.

of a remarkable quantity having a greater amount in methanolic extract than that of aqueous extract (Table 1). Several reports have revealed that brown algae contain a higher amount of polyphenols than green and red algae. Also, the phenolic and flavonoid contents are more soluble in polar solvents like methanol, ethanol, and acetone. From earlier reports, it is evident that phlorotannins are bipolar and are mostly found in brown algae (30). Probably, they contribute to the higher flavonoid content in the methanolic extract of *S. wightii* as observed in this study. AA was found in a higher amount in the aqueous extract of *S. wightii* (Table 1).

DPPH is a stable nitrogen-centered free radical. When a DPPH solution is mixed with a substrate acting as a hydrogen atom or electron donor, its color changes from violet to yellow. The substances capable of showing this reaction are considered antioxidants or radical scavengers. DPPH scavenging activity is a widely used model to screen antioxidant activity of plants and mostly this property of plant extracts is a function of their phenolic contents (31). In this work, the higher DPPH scavenging activity of methanolic extract of *S. wightii* can be attributed to its high phenolic content.

Hydroxyl radical, the most active ROS can cause oxidative damage to DNA, lipids, and proteins contributing to cytotoxicity. In our investigation, *Sargassum* extracts possessed hydroxyl radical scavenging activity as measured by deoxyribose assay. Our findings are in agreement with that of a previous work reporting similar results with *Sargassum siliquosum*. *S. wightii* extracts. They also revealed SOD scavenging activity but with a higher  $\text{IC}_{50}$  in comparison to that of AA (20) In ferric acid-reducing antioxidant power (FRAP) assay, the antioxidant activity was determined as a function of the reducing capacity of the test sample from ferric (III) to ferrous (II) in a redox-linked colorimetric reaction (30). The reducing power shown by *S. wightii* extracts in this

work is believed to be the strong heavy metal chelating activity due to their phenolic and flavonoid components. A similar corroboration was made by Thilina et al (32). Girija et al have correlated the phlorotannin content of *Turbinaria ornata* (brown seaweed) with its strong superoxide and DPPH scavenging activity (33).

The role of the NO free radical in the inflammatory processes involving many pathological conditions including cancer is well documented. In this experiment, the use of the Griess reaction explains the NO radical scavenging activity of plant extracts that prevent the formation of NO. Thus, the dysfunction of NO generating cells like endothelial cells, macrophages, and neurons could be prevented by the use of *Sargassum* extracts. A similar correlation was reported by Wijesoorya et al (34).

Most of the biological Proteins get denatured by the application of stress or chemicals and lose their function. Protein denaturation is an important cause of inflammation (35). Hence, the anti-inflammatory potential of the *Sargassum* extracts was studied and observed that both methanolic and aqueous extracts inhibited protein denaturation (albumin). It is well documented that serine proteinase contained in neutrophils plays an important role in inflammatory reaction during tissue damage, and proteinase inhibitors impart significant protection (36). In our observation, both *S. wightii* extracts possessed antiprotease activity explaining their anti-inflammatory potential (Table 2).

In our observation, the aqueous extract of *S. wightii* exhibited a lower antioxidant activity than that of methanolic extract having higher  $\text{IC}_{50}$  values. The antioxidant activity of aqueous extract could be possibly due to the ascorbic content and hydrophilic polyphenols, peptides, fucoidan, and Millard reaction products (37). The antioxidant activity shown by methanolic extract might be attributed to its high phenolic contents. The antioxidant activities of brown seaweed from various

parts of the world have been reported. Many of them are in accordance with our observations and some are contradicting. The difference in various parameters among *Sargassum* weeds might be due to different geographical distribution, methods of extraction, and the solvents used for extraction and preservation.

The antioxidant and anti-inflammatory activities observed in this study suggest the use of *S. wightii* as a natural source of antioxidants and anti-inflammatory agents. However, this work lacks the isolation of different active principles in all solvent extracts and assessment of antioxidant and anti-inflammatory activities in various test models, which needs future research.

### Conclusion

This study results showed that *S. wightii* has a significant antioxidant and anti-inflammatory potential. These useful pharmacological activities show the potential benefits of this plant as a lead compound for designing a new drug that could have a protective role against conditions like cancer, neurological disorders, diabetes, cardiovascular diseases, aging, and inflammation.

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### Authors' contributions

BR designed and guided the study. SR followed the research idea and carried out the experiment by following standard experimental guidelines, provided the data, and wrote the draft of the manuscript. AK and IR carried out the literature review and statistical analysis and helped in the result interpretation. SKB supervised the study. All authors reviewed and approved the final manuscript

### Conflicts of interest

Authors declare that there are no conflicts of interest.

### Ethical considerations

All the experiments confirmed and performed as per the Roland Institute of Pharmaceutical Sciences institutional ethical standard experimental guidelines. Ref. ID: Eastern/7004019843/2020/RIPS.

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