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In vitro Anti-Tumor, Anti-Inflammatory, Antioxidant and Antibacterial Activities of Marine Brown Alga *Sargassum wightii* Collected from Gulf of Mannar

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Abstract: *Sargassum sp.,* growing in tropical and sub tropical regions have been known for a wide range of biological activities. In the present study, the antitumor, anti-inflammatory, antioxidant and antibacterial activities of crude methanolic extract of *Sargassum wightii* Greville was investigated *in vitro*. The chemical constituents of active fractions were analyzed by gas chromatography-mass spectrometry (GC-MS). Preliminary screening revealed that the methanol extract of *S. wightii* inhibited the proliferation of human colon carcinoma cell line HT-29 at IC_{50} 0.13 mg/ml and peripheral blood mononuclear cells (PBMCs) at IC_{50} 74.77 µg/ml. It also exhibited strong scavenging activity on DPPH and superoxide radicals at IC_{50} 1.07 and 1.01 mg/ml. Reducing power and total antioxidant level increased with increasing extract concentration. Crude methanol extract inhibited the growth of *Bacillus cereus* at MIC 50 µg/ml and other Gram-negative pathogens at 100 µg/ml except *Escherichia coli* (110 µg/ml). GC-MS analysis of active fractions III and VI revealed the presence of major groups of compounds from hydrocarbons, esters, ketones and other miscellaneous compounds.

Key words: *S. wightii* · Anti-Inflammatory · Antioxidant · Anti-Cancer · GC-MS

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated. Reports on the uses of seaweeds have been cited as early as 2500 years ago in Chinese literature [1]. The long history of seaweed utilization for a variety of purposes has led to the gradual realization that some of their constituents are superior and more valuable in comparison to their counterparts on land. Use of seaweeds as potential producers of pharmaceutical products has been reviewed [2]. Recently, a great deal of interest has developed to isolate bioactive compounds from marine resources because of their numerous beneficial health effects [3].

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries [4]. Colon cancer is one of the most malignant neoplasia in the world and linked with dietary habits such as high animal fat intake. Inflammation is a tissue reaction to infection, irritation or foreign substances have linked with pathogenesis of many diseases such as cancer, neurodegenerative diseases, arthritis and other metabolic disorders [5]. Seaweeds have attracted special interest as good sources of anti-inflammatory agents. Earlier studies on anti-inflammatory activities of *Sargassum sp.* have been reported [6, 7].

Reactive oxygen species (ROS) is generated in living organisms in the forms of superoxide anion (O_2^-) Hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and nitric oxide (NO). Excessive generation of ROS initiates biomolecular oxidations and creates oxidative stress, which leads to age related degenerative diseases. The negative effects of oxidative stress may be mitigated by antioxidants [8]. Haphazard use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds. Marine algae extracts have been reported for strong antioxidant

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properties [9, 10]. They do this by preventing the generation of free radical and ROS, or by activating a battery of detoxifying proteins. Hence, attention towards natural antioxidants has been the focus of present research to replace synthetic antioxidants.

Sargassum is a tropical and sub-tropical brown seaweed comprising 150 species, common to all oceans except Antarctica [11]. The chemical composition of *Sargassum* has been studied extensively and isolation of phlorotannins [12], phlorethols [13], sterols [14] and dicotylpthalate [15] have been recognized. Though literature speaks miscellaneous studies on bioactivity of Sargassum species, information remained lacking about the *in vitro* antioxidant, anti-inflammatory and anticancer activities of *Sargassum wightii* Greville. This paucity of knowledge initiated the current investigation on characterization of antitumor and anti-inflammatory activity on human colon carcinoma cell line HT-29 and human peripheral blood mononuclear cells by MTT (3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) assay. The antioxidant potential of brown seaweed *S. wightii* by DPPH (2, 2-diphenyl-1-picrylhydrazyl), superoxide radical-scavenging assay, total antioxidant and reducing power assay was also carried out. Purified fractions of crude extract were analyzed by GC-MS for the identification of chemical constituents.

MATERIALS AND METHODS

Plant Material: Fresh seaweed samples of *S. wightii* were collected from Mandapam, (Tamilnadu), India during December 2013. Plant specimen was identified by Prof. N. Parthasarathy, Salim Ali School of Ecology, Pondicherry University. Specimen was preserved in 5% formalin solution for identification.

Preparation of Extracts: The seaweed sample was cleaned with seawater three times and then consecutively with tap water and distilled water to confiscate the epiphytes and other wastes. Finally, the sample material was desiccated under the shade for two weeks. The desiccated plant materials (10 g dry weight) were ground to fine powder and extracted with 100 ml of methanol for 24 h using a Soxhlet extraction apparatus and the extract was sifted through a Buchner funnel with Whatman No. 1 filter paper. This was repeated three times for the complete extraction of compounds and all three solvent extracts were pooled. The solvent was evaporated from crude extract by rotatory evaporator. The dried extract (1 g) was dissolved in 2 ml of respective solvent and stored at 4°C until use.

Anticancer Assay: The human colon carcinoma cell line HT-29 was procured from National Centre for Cell Sciences, Pune and maintained with DMEM medium containing 10% fetal bovine serum and 100 ng/ml, each, of penicillin and streptomycin at 37°C in a humidified atmosphere with 5% CO_2.

Growth Inhibition Assay: The inhibition effect of *S. wightii* methanol extract on the growth of HT-29 cells was evaluated *in vitro* by MTT assay. Briefly, the HT-29 cells (5×10^4 cells/ml) were incubated in 96-well plates containing 100 µl of the culture medium at 37°C in a humidified atmosphere with 5% CO_2. Cells were permitted to adhere for 24 h, then washed with 100 µl of phosphate-buffered saline (PBS). Different concentrations of extract (0.0625, 0.125, 0.250, 0.500, 1.000 mg/ml), 100 µl each, were added to each well. After 72 h of exposure, the extract containing medium was removed, washed with 100 µl of PBS and replaced by fresh medium. The cells in each well were then incubated in culture medium with 20 µl of MTT (5 mg/ml) for 4 h. After the media were removed, 100 µl of DMSO was added to each well. Absorbance at 570 nm was determined by Triad multimode detector. The inhibition rate (IR) was calculated according to the formula:

Growth inhibition rate (%) = (Absorbance of test sample /Absorbance of control) × 100.

Anti-Inflammatory Assay: The anti-inflammatory assay was carried out as described earlier [16]. Briefly, peripheral blood mononuclear cells were isolated from human blood by Histopaque-1077 (Sigma) density gradient using standard procedures. PBMCs from the buffy layer were pipetted out and finally suspended in RPMI–1640 medium (Sigma) containing 10% fetal calf serum. Total number of cells were counted and assessed for cell viability using trypan blue exclusion test.

Mitogen Induced Lymphocyte Proliferation and its Inhibition by Anti-inflammatory Agents: PBMCs of (2×10^5 cells/well) were seeded in a volume of 200 µl in RPMI–1640 medium containing 10% FCS and 1 µg/ml of phytohemagglutinin (PHA) in a 96–well U bottom plate

trailed by the addition of anti-inflammatory agent at various concentrations. The compound treated cells were incubated for 24 h in CO_2 incubator at 37°C containing 5% CO_2 and 90% humidity. Assays were performed in triplicate for each concentration of seaweed extract. Experimental data represent mean ± SD of each compound, unless otherwise stated.

Cytotoxic Studies by MTT Assay: To confirm the suppressive effect of crude methanol extract of seaweed on lymphocyte proliferation, 2 µl of the compound at various concentrations (1, 10, 50, 100 µg/ml) was added. Triton X served as negative control, DMSO was used as solvent vehicle. Subsequent removal of medium from the wells, 10 µl of MTT (5 mg/ml resuspended in PBS) was added to each well and incubated at 37°C for 4 h. After 4 h, the floating cells were carefully removed and 50 µl of DMSO was added to each well to lyse the cells. Absorbance was measured at 570 nm using a microplate reader. The percent cell viability was calculated as follows.

$$\text{Cell viability (\%)} = (A_{test}570 / A_{cont}570) \times 100$$

where, A_{test} was the absorbance in the presence of compound at various concentrations and A_{cont} was the absorbance of the control.

Antioxidant Assays

DPPH Radical Scavenging Activity: The scavenging effect of seaweed extract was determined by the method of Blois, [17]. Briefly, 150 µl of DPPH solution was added to 3 ml methanol and the absorbance was taken immediately at 516 nm for control reading. A test sample at various concentrations (0.2-1.0 mg/ml) was mixed with 3 ml of methanol and 150 µl of DPPH (0.1 mM) solution. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the resulting mixture was measured at 516 nm in a UV-visible spectrophotometer (U-2000 model, Hitachi, Japan). Decrease in absorbance of reaction mixture indicated a high free radical scavenging activity. Butylated hydroxyanisole (BHA) was used as reference compound. Assays were performed at least in triplicate. The percent DPPH scavenging effect was calculated as follows.

$$\text{DPPH Scavenging effect (\%)} = (A_{cont} - A_{test} / A_{cont}) \times 100.$$

where, A_{cont} was the absorbance of the control reaction and A_{test} was the absorbance of the seaweed sample. Experimental data represent mean ± SD of seaweed sample, unless otherwise stated.

Superoxide Anion Scavenging Activity: The superoxide anion scavenging ability of seaweed extract was assessed based on the method described by Nishikimi *et al*. [18]. Superoxide radicals were generated in 3 ml Tris-HCl buffer (16 mM, pH 8.0) containing 300 µM NBT, 468 µM NADH and sample at different concentrations (0.2-1.0 mg/ml). The reaction was started by adding 60 µM PMS to the mixture. The reaction mixture was then incubated at room temperature for 5 min and the absorbance was read at 560 nm against the blank. In the control, sample was substituted with Tris-HCl buffer. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. Gallic acid was used as reference compound. Assays were performed at least in triplicate. The capability of scavenging superoxide radical was calculated using the following equation.

$$\text{Scavenging effect (\%)} = (1-A_{test}\ 560/A_{cont}560) \times 100$$

where, $A_{cont}560$ was the absorbance of the control reaction and $A_{test}560$ was the absorbance of the seaweed sample. Experimental data represent mean ± SD of seaweed sample, unless otherwise stated.

Total Antioxidant Activity: Total antioxidant activity of seaweed extract was determined by the method of Prieto *et al*. [19]. Briefly, 3.0 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4.0 mM ammonium molybdate) was added to the test tube containing 0.3 ml aliquot of sample at different concentrations (0.5-2.5 mg/ml). The reaction mixture was then incubated at 95°C for 90 min under water bath. After incubation, absorbance of all the sample mixtures was measured at 695 nm. Ascorbic acid was used as reference compound. Assays were performed at least in triplicate. Increase in absorbance of the reaction mixture the superior is the total antioxidant activity. Experimental data represent mean ± SD of seaweed sample, unless otherwise stated.

Reducing Power: Reducing power of seaweed extract was determined by the method of Oyaizu [20]. Briefly, 1.0 ml of crude methanol extract of seaweed at different

concentrations (100-500 µg/ml) was mixed with phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The reaction mixture was incubated at 50°C for 20 min. Subsequent to incubation, 2.5 ml of 10% trichloroacetic acid was added to the reaction mixture to stop the reaction which was centrifuged at 1,000 × *g* for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml), $FeCl_3$ (1.5 ml, 0.1%). Absorbance of all the sample solutions was measured at 700 nm. Assays were performed at least in triplicate. Ascorbic acid at the aforementioned concentrations was used as standard. Increased absorbance indicates greater the reducing power. Experimental data represent mean ± SD of seaweed sample, unless otherwise stated.

Antibacterial Assay

Test Organisms: Bacteria, *Vibrio parahaemolyticus* MTCC 451, *V. fischeri* MTCC 1738, *V. vulnificus* MTCC 1145, *Bacillus cereus* MTCC 430 and *Escherichia coli* MTCC 1687 were acquired from Microbial type culture collection, IMTECH, Chandigarh, India. *V. anguillarum* was acquired from Central Institute of Brackish-water Aquaculture (CIBA), Chennai. The multidrug resistant bacteria *Acinetobacter baumannii* was procured from Pondicherry Institute of Medical Sciences (PIMS), Pondicherry, India and the strain was biochemically characterized [21]. The pathogenic bacteria were cultured independently on Tryptic soy broth at 37°C for 18 h, before inoculation for assay. Broth culture (100 µl), which contained 10^7-10^8 number of bacteria per ml was appended to tryptic soy agar medium (Hi-media, Mumbai), decanted into sterile Petri dishes and allowed to solidify.

Growth Inhibition: Growth inhibition of pathogens by seaweed extract was appraised using the paper disc diffusion method. Briefly, sterile filter paper discs impregnated with crude extract (200 µg/ ml), positive control (Ampicillin 50 µg/ml) and negative control (corresponding vehicle) were allowed to dried out and subsequently laid equidistantly onto the surface of the pathogen seeded tryptic soy agar plates. The plates were reserved in an upturned position and incubated at 37°C for 18 h. The growth inhibition was measured as the diameter (in mm) of the zone of inhibited microbial growth. The experiment was carried out in triplicate. Experimental data represent mean ± SD of each sample, unless otherwise affirmed.

Minimum Inhibitory Concentration (MIC) Assay: A broth microdilution method was employed to determine the Minimum Inhibitory Concentration [22-24]. All tests were carried out in the Mueller-Hinton agar medium (Hi-media).

Purification of Fractions by Thin Layer Chromatography: To examine the chemical components, concentrated crude methanol extract of seaweed was purified by thin layer chromatography using methanol-chloroform as solvent systems. The crude extract was separated in a pre coated aluminum TLC sheet with silica gel G 60 as stationary phase and methanol-chloroform mixture in the ratio of 10:1 as mobile phase. The eluted spots, representing various fractions were investigated under UV transilluminator at 254 nm and also in the iodine chamber. TLC resolved spots of methanol extract at various R_f values were scrapped out from the TLC plate and the scrapped spots were dissolved in methanol, mixed well and centrifuged at 12,000 × *g* for 5 min. A total of six different fractions were collected and the supernatant (40 µl) of each fraction was used to ensure the antibacterial activity against pathogens using the disc diffusion method in triplicate. Ampicillin was used as positive control. Experimental data represent mean ± SD of each sample, unless otherwise stated.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: The purified fractions were analyzed for chemical constituents following the same method as described earlier [16]. Briefly, 2 µl sample volume was injected into the column and ran using split less mode. After 2 min, the oven temperature was raised to 150°C at a ramp rate of 10°C/min. The oven temperature was then raised to 250°C at a ramp rate of 5°C/min and finally the oven temperature was raised to 280°C at a ramp rate of 20°C/min and maintained at this temperature for 40 min. The helium carrier gas was programmed to maintain a constant flow rate of 1 ml/min and the mass spectra were acquired and processed using both Agilent ChemStation (Agilent, USA) and AMDIS32 software. The major compounds were identified by comparison of their mass with authentic standards.

Statistical Analysis: All results were expressed as mean ± standard deviation. The means of all the parameters were examined for significance by one way analysis of variance (ANOVA) and differences were considered to be

statistically significant if $p < 0.05$. Tukey's *post hoc* test was performed for multiple group comparison between concentrations. All computations were carried out by employing statistical software (SPSS Version 7.0).

RESULTS AND DISCUSSION

In recent years, pharmaceutical firms have started gazing towards marine organisms, including seaweeds, for new drugs from natural products [25]. Many chemically unique compounds of marine origin with diverse biological activities have been isolated and or are being developed as new pharmaceuticals [2]. Cancer is a formidable problem for people. To investigate the antitumor activity of *S. wightii* crude extract *in vitro*, the human colon carcinoma cell line HT-29 treated with different concentrations of extract and inhibitory effect was depicted (Fig. 1). The present study showed that, *S. wightii* methanol extract could extensively inhibited cell proliferation architecture in dose dependent manner and fifty percent of inhibition was observed at IC_{50} 0.13 mg/ml. Similarly, ethyl acetate extract of brown seaweed *S. thunbergii* demonstrated cytotoxic effect greater than 50% on HT-29 cell lines at 0.10 mg/ml [26]. To the best of our knowledge, this is the first report demonstrating the *in vitro* anticancer activity of the methanol extract of *S. wightii* providing a scientific basis for its effects on human health.

Anti-inflammatory activity has been reported from several marine plants of different families [27, 28]. The anti-inflammatory activity of different solvent extracts of *S. wightii* has been documented *in vivo* collected from North Arabian Coast of Pakistan [29]. In the present study, the *in vitro* anti-inflammatory activity of seaweed *S. wightii* and its effect at various concentrations on the inhibition of proliferation of mitogen induced PBMCs was investigated and portrayed (Fig. 2). There was significant increase in proliferation of PBMCs on induction with PHA but this response was considerably inhibited by crude methanol extract from *S. wightii*. Fifty percent inhibition (IC_{50}) of proliferation of PBMCs was observed at 74.77 µg/ml concentration of crude methanol extract. The anti-inflammatory potential of *S. wightii* extract on proliferation of mononuclear cells, which are crucial for generation of effective immune responses, was analyzed in the presence of the mitogen PHA. Butanol and hexane extracts of *S. wightii* collected during winter season showed relatively higher inhibition compared to the methanol extract of carrageenan induced rat paw edema [29]. Interestingly, the methanol extract of *S. wightii* collected during winter season inhibited the PBMC proliferation under *in vitro* condition. Similar results were observed in organic extracts from different plants [30, 31]. For the first time, this finding reinforced that the crude methanol extract of seaweed *S. wightii* do contain compounds, capable of suppressing PBMC proliferation. Further study is in need to justify the suppression of proliferation of PBMC is by means of cytotoxic effects of this methanol extract. Our preliminary results encourage us to further investigate the detailed study on anti-inflammatory potential of these crude extract that may lead to promising compounds.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Therefore, DPPH have been used extensively as free radicals to evaluate reducing substances and are useful reagent for investigating the free radical scavenging activities of compounds. Methanol extract of *S. wightii* showed significant DPPH radical scavenging activity ($p < 0.05$) between concentrations and IC_{50} was observed at 1.07 mg/ml (Fig. 3). Similarly, the boiling extract of *Sargassum* species scavenged the DPPH radicals at IC_{50} of 1.08 ± 0.83 mg/ ml [32]. Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxidative species. The superoxide radical was assayed in the reduction of NBT that was being generated in a PMS/NADH system [33]. In this study, superoxide radicals scavenged by methanol extract of *S. wightii* was found to be statistically significant ($p < 0.05$) and scavenging activity at 50% was observed at the concentration of 1.01 mg/ml (Fig. 4). However, most extracts acquired with proteases and carbohydrases did not exhibit superoxide radical scavenging activity ranging from 10% to 20% at 1 mg/ml [34]. This present finding strongly supported by the existing reports in other higher plants including brown and red seaweeds [9]. The scavenging activities were found to be increased with increasing concentration of extract in DPPH in addition to superoxide assays. In effect, the inhibitory capacity of the methanol extract was inferior to those of the commercial counterparts such as butylated hydroxyanisole and gallic acid.

Total antioxidant activity of methanol extract of *S. wightii* was depicted in Fig. 5. In this phosphomolybdenum method, a green phosphate/Mo (V) complex is formed by the reduction of Mo (VI).

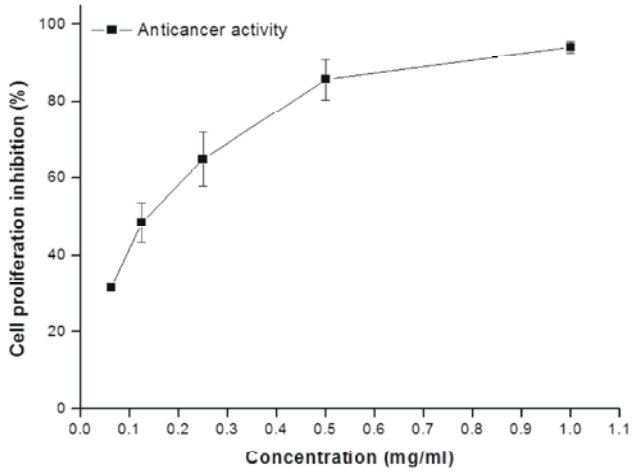

Fig. 1: Growth inhibition activity of methanol extract from *S. wightii* on human colon carcinoma cell line HT-29. Results were representative of three separated experiments

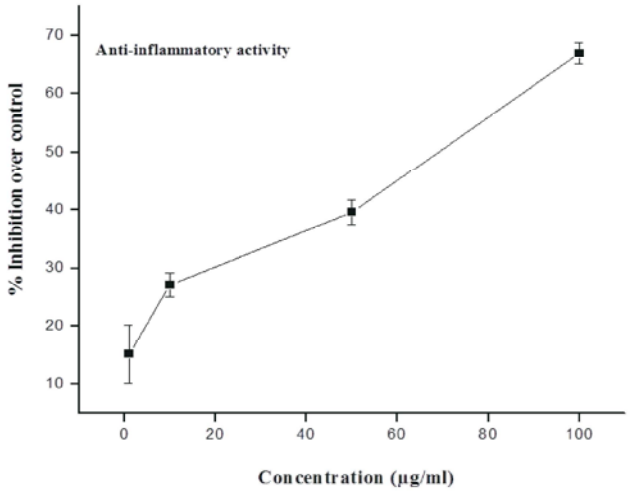

Fig. 2: Inhibitory effect of crude extract from *S. wightii* on mitogen induced proliferation. Mitogen PHA (1 µg/ml) induced PBMC (2×10^5/well) was treated with different concentration of crude extracts and the % inhibition of lymphocyte proliferation was determined over control. Results were representative of three separated experiments

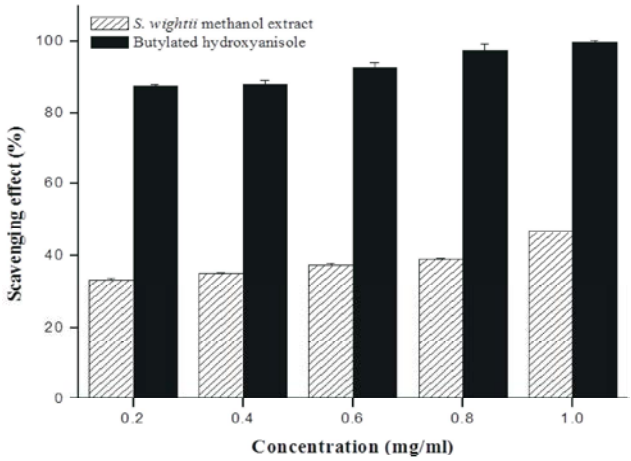

Fig. 3: DPPH radical scavenging activity (%) of methanol extract obtained from of seaweed *S. wightii* at different concentrations. Results were representative of three separated experiments

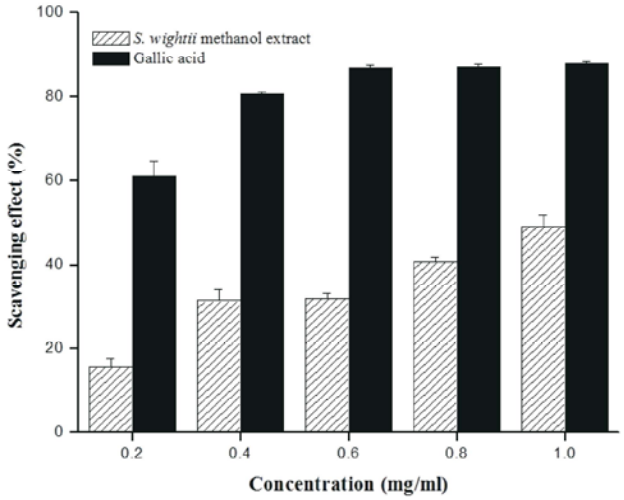

Fig. 4: Superoxide radical scavenging activity (%) of methanol extract obtained from of seaweed *S. wightii* at different concentrations. Results were representative of three separated experiments

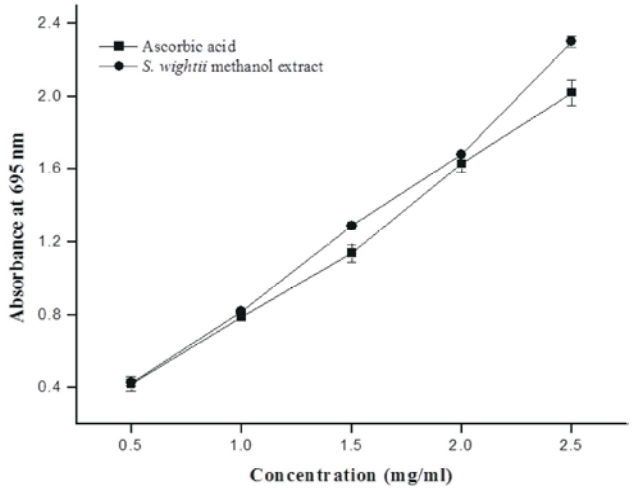

Fig. 5: Total antioxidant activity of *S. wightii* methanol extract. Results were representative of three separated experiments

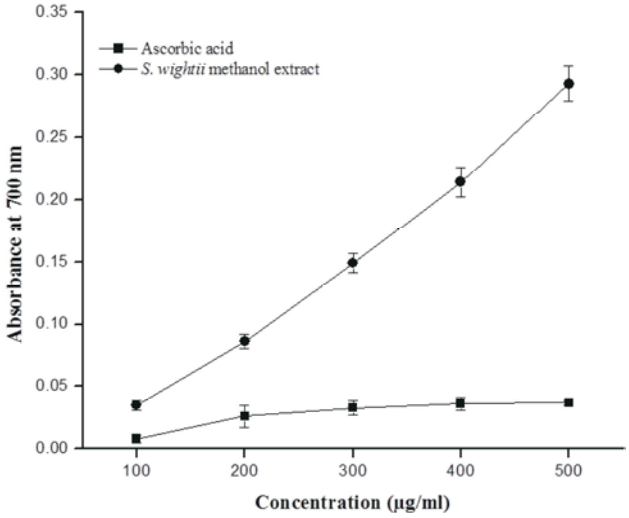

Fig. 6: Reducing power of *S. wightii* methanol extract. Results were representative of three separated experiments

The total antioxidant activity increased with increase in absorption from 0.43 ± 0.019 to 2.30 ± 0.032 at increasing concentration (0.5-2.5 mg/ml). The sample was statistically significant ($p < 0.05$) as compared to commercial counterpart ascorbic acid at 0.5 mg/ml. The present study corroborated well with existing reports [35]. It has been reported that antioxidant activities and reducing power has a direct correlation with each other [36]. The reducing power of *S. wightii* methanol extract was shown in Fig. 6. As shown in the figure, the reducing power of sample correlated well with increasing concentration. The absorption was increased from 0.035 ± 0.004 to 0.293 ± 0.014 with the concentration increased from 100-500 µg/ml. Correspondingly, the reducing power increased in positive control (ascorbic acid) from 0.008 ± 0.003 to 0.037 ± 0.002 with increasing concentration. Furthermore, it was examined that methanol extract of *S. wightii* had greater reducing power than ascorbic acid at each concentration point. The reducing capacity of *S. wightii* methanol extract was statistically significant ($p < 0.05$) as equated to ascorbic acid. However, both the sample and ascorbic acid showed significant difference between the concentrations ($p < 0.05$). This property is associated with the presence of reductones that are reported to be terminators of free radical chain reaction. However, the reducing power of *S. marginatum* (C. Agardh) J. Agardh (Sargassaceae) was found to be less even at 1 mg/ml concentration [37]. The present finding showed that methanol extract of *S. wightii* exhibited strong reducing power than earlier reports.

Several species of seaweeds have caused an emerging avenue in the biomedical field due to their great potential as antimicrobial, anti-inflammatory, antiviral and anti-tumoral drugs [25, 38]. In the present study, the methanol extract of seaweed *S. wightii* collected from Mandapam, Rameshwaram coastal line exhibited antibacterial activity against Gram-positive *Bacillus cereus* and Gram-negative human pathogens. The results of the antibacterial activity of crude extract and minimum inhibitory concentration against Gram-positive and Gram-negative pathogens were summarized in Table 1. In this study, the methanol extract of *S. wightii* (200 µg/ml) exhibited a good antibacterial activity of 18 mm against *Bacillus cereus* followed by 15 mm against *Acinetobacter baumannii*. The growth of *Bacillus cereus* was inhibited at a minimum inhibitory concentration of 50 µg/ml followed by Gram-negative pathogens at 100 µg/ml. Whereas, *E. coli* growth was inhibited at a minimum inhibitory concentration of 110 µg/ml. The results of the present study corroborated well with some preceding studies of seaweeds antibacterial activity against fish pathogens [39]. Prior reports showed that methanol extract of *S. wightii* inhibited the Gram-positive and Gram-negative pathogens which validated the present study. However, variation of antibacterial activity of our extract might be due to the presence of antibacterial substances, which could be varied from algal species to species [40]. Conflicting reports were observed on the presence of bioactive compounds in the seaweeds related to the seasonal variation, as well as the method of extraction and organic solvents used for extraction of bioactive compounds and differences in assay methods [41]. In the present study, the methanol extract of *S. wightii* controlled the growth of *B. cereus* at a minimum concentration of 50 µg/ ml. Similarly, crude methanol extract of *S. wightii* was effective in controlling the growth of *Pseudomonas syringae* at the minimum inhibitory concentration of 50 µg/ml [42].

The antibacterial activity of TLC purified fractions of seaweed *S. wightii* was depicted in Table 2. Purified fraction III showed the inhibition zone of 11 mm against *B. cereus* and 10 mm against the other pathogens tested except *E. coli* (6 mm). Similarly, purified fraction VI showed the maximum inhibition zone of 13 mm against *B. cereus, A. baumannii* (12 mm), *V. anguillarum, V. parahaemolyticus* (11 mm) and *V. fischeri, V. vulnificus* (10 mm). Whereas, diameter of less than 10 mm was observed against *E. coli* (8 mm). Whereas, diameter of less than 10 mm was observed in other fractions against the pathogens tested. In this study, the purified fractions of seaweed *S. wightii* displayed good antibacterial activity against Gram-positive as well as Gram-negative pathogens. Fractions III and VI of *S. wightii* extract efficiently inhibited *B. cereus, A. baumannii, V. parahaemolyticus* and *V. anguillarum*. Similar result was observed from purified fractions of selected South African seaweeds against Gram-positive as well as Gram-negative pathogens [43]. The antibacterial activity of marine algae and mangrove plants against fish pathogens was observed and the fractions of methanol extract of red seaweed *Gracilaria corticata* J. Agardh (Gracilariaceae) showed good activity against fish pathogens *Pseudomonas aeruginosa* and *V.* alginolyticus [39].

The results obtained by the GC-MS analysis of the purified fractions III and VI are presented in the Table 3 and 4. As determined from the GC-MS analysis, the major compounds were 9- Octadecenamide (11.40%), Eicosane (6.47%), Hexanoic acid, pentadecyl ester (5.57%), 1H-Indole, 6-methyl-(5.43%), Estra-1, 3, 5 (10)- trien- 17.beta.-

Table 1: Antibacterial screening of crude extract of different solvents of seaweed *S. wightii* against pathogens. (inhibition zone was measured to nearest millimeter)

	Inhibition zone in mm (mean ± SD)		
Pathogens	Crude extract (200 µg/ml)	MIC (µg/ml)	Ampicillin (50 µg/ml)
V. parahaemolyticus	10.83±0.76	100	12.00±0.57
V. anguillarum	10.16±1.04	100	10.00±0.00
V. fischeri	11.83±0.76	100	13.00±0.50
V. vulnificus	12.16±1.04	100	12.16±0.28
E. coli	08.33±0.76	110	12.00±0.50
B. cereus	17.83±0.76	50	10.16±0.28
A. baumannii	15.16±1.25	100	10.16±0.28

Values are means of growth inhibition of three replicates.

Table 2: Antibacterial screening of minimum inhibitory concentration and purified fractions of methanol extract of seaweed *S. wightii* against pathogens (inhibition zone was measured to nearest millimeter)

	Inhibition zone in mm (mean ± SD)					
Pathogens	Fr-1	Fr-2	Fr-3	Fr-4	Fr-5	Fr-6
V. parahaemolyticus	00.00±0.00	00.00±0.00	10.00±0.57	06.00±0.57	08.00±0.57	11.00±0.57
V. anguillarum	00.00±0.00	00.00±0.00	10.00±0.16	00.00±0.00	08.00±0.57	11.00±1.52
V. fischeri	00.00±0.00	00.00±0.00	10.00±0.57	06.00±0.57	09.00±0.16	10.00±0.57
V. vulnificus	08.00±0.57	00.00±0.00	10.00±0.57	07.00±0.00	08.00±0.57	10.00±0.57
E. coli	00.00±0.00	00.00±0.00	06.00±0.57	00.00±0.00	00.00±0.40	08.00±0.57
B. cereus	06.00±0.57	07.00±0.57	11.00±0.57	07.00±0.57	08.00±0.57	13.00±0.57
A. baumannii	00.00±0.00	06.00±0.57	10.00±0.57	00.00±0.00	00.00±0.00	12.00±0.57

Values are means of growth inhibition of three replicates, Fr= fraction

Table 3: GC-MS analysis of fraction III of *S. wightii*

Rt (min)	Compound	Area (%)
30.57	Eicosane	06.47
30.78	Hexanoic acid, pentadecyl ester	05.57
31.28	Butane, 2, 3-bis (trimethylsiloxy)-	03.29
31.37	1H-Indole, 6-methyl-	05.43
32.57	Estra-1, 3, 5 (10)- trien- 17.beta.-ol	03.52
32.87	9- Octadecenamide	11.40

Table 4: GC–MS analysis of fraction VI of *S. wightii*

Rt (min)	Compound	Area (%)
18.90	1, 2- Benzenedicarboxylic acid, butyl 1 octyl ester	03.54
30.84	26-Nor-5- cholesten-3.beta.-ol-25-one	14.55
31.38	18-Crown-6	05.26
31.83	1, 4, 7, 10, 13, 16- Hexaoxacyclooctadecane	05.98
32.45	1, 4, 7, 10, 13, 16-Hexaoxanonadecane, 18- (2-propenyl)	10.52
32.58	7- hydroxyl-3-(1,1-dimethylprop-2-enyl) coumarin	05.50
32.88	9- Octadecenamide	07.95
33.27	Methylenebis (2, 4, 6-triisopropylphenylphosphine)	09.47

Rt = Retention time

ol (3.52%) and Butane, 2, 3-bis (trimethylsiloxy)-(3.29%) in purified fraction III. Whereas, fraction VI revealed the presence of 26-Nor-5- cholesten-3.beta.-ol-25-one (14.55%), 1, 4, 7, 10, 13, 16-Hexaoxanonadecane, 18- (2-propenyl)-(10.52%), Methylenebis (2, 4, 6-triisopropylphenylphosphine) (9.47%), 9- Octadecenamide (7.95%), 1, 4, 7, 10, 13, 16- Hexaoxacyclooctadecane (5.98%), 7- hydroxyl-3-(1,1-dimethylprop-2-enyl) coumarin (5.50%), 18-crown-6, (2-bromophenyl)- (5.26%), 1, 2-Benzenedicarboxylic acid, butyl 1 octyl ester (3.54%) (Table 4). There are numerous reports of compounds derived from macro algae with a broad range of biological activities such as antibiotics [44] and hydrocarbon [45]. The GC-MS analysis of active fractions of *S. wightii* in

this study revealed the presence of major groups of compounds from hydrocarbon, esters, ketones and other miscellaneous compounds. Similar group of compounds were reported in brown seaweeds *Kappaphycus alvarezii* (Doty) and *S. polycystum* C. Agardh [45] and in essential oils of *Allium atroviolaceum* Boiss (Alliaceae) flowers [46].

CONCLUSION

The results of the present study showed that methanol extract of *S. wightii* may offer potential for use as antibacterial, antioxidant and anti-inflammatory candidate. GC-MS analysis of purified fractions revealed the presence of hydrocarbons, ketones and esters. Further investigations on *S. wightii* can provide a more efficient way of discovering novel chemicals with unique pharmacological properties or biomedical uses. However, the abundant availability of seaweeds along the Indian coastline remains a mystery for the entry of pharmaceutical industries.

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