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Antioxidant, Antimicrobial and Cytotoxic activity of Some Common Seaweed along West Coast of Maharashtra

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ABSTRACT

This study aimed to evaluate the antioxidative potential and antimicrobial activity of eleven seaweeds, including *Sargassum ilicifolium*, *Sargassum tenerrimum*, *Stoechospermum marginatum*, *Padina tetrastromatica*, *Dictyota maxima*, *Gracilaria corticata*, *Acanthophora spicifera*, *Batrachospermum* sp., *Caulerpa peltata*, *Chaetomorpha crassa*, *Ulva lactuca*. *Sargassum ilicifolium*, *Sargassum cinereum*, and *Sargassum tenerrimum* were studied for anticanser activity. Seaweed extracts were screened for their antimicrobial activity against the following pathogens: *Klebsiella*, *Bacillus thrungenis*, *Psudeomonus* sp., and *Streptococ*cus *aureus*. Antimicrobial activity was measured using ELISA microplate reader. In ethanol extract, *Sargassum tenerrimum* and *Caulerpa peltata* exhibited maximum inhibition on the growth of the tested *Klebsiella* species. The antioxidant potential of various seaweed extracts was determined using the DPPH and FRAP. In the present study, the DPPH activity was consistently higher in the methanolic extracts of Phaeophyta. The maximum percentage of radical-scavenging activity was observed in the *Padina tetrastromatica*. This study was designed to assay cytotoxic activity of the extract from brown alga; namely, *Sargassum ilicifolium*, *Sargassum cinereum* and *Sargassum tenerrimum* against breast cancer cells MCF7 by using MTT assay. The research was performed as an in vitro study. The *Sargassum cinereum* showed the highest IC50 value compared to *Sargassum ilicifolium* and *Sargassum tenerrimum.* The seaweed extract can be used as promising anti-oxidative agents, anti-microbial and anti-cancerous in cosmeceutical or nutraceutical products which can upturn the value of an otherwise worthless weed.

INTRODUCTION

Humans are impacted by free radicals generated either inside the body or from the surrounding environment by reactive oxygen species (ROS). ROS, which includes superoxide (O2-), hydroxyl (HO•), and hydrogen peroxide(H_2O_2), may also be developed by so many biochemical activities in the cells (endogenous sources). Ionizing radiation,

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UV light, tobacco smoke, toxic waste, and toxins; all are examples of exogenous sources of reactive oxygen species. The protective action of antioxidants presented in tissues will help to minimize ROS and free radicals **(Halliwell & Cross, 1994)**. The antioxidant defence mechanism ensures that fairly constant levels of ROS are retained in cells. However, under stressful conditions, this prickly balance is disrupted, resulting in increased ROS development **(Betteridge, 2000)**. These ROS can harm important bio-molecules such as proteins, DNA and lipids, resulting in a variety of human diseases such as atherosclerotic **(Wu *et al.*, 1998)**. Lipid oxidation is a common occurrence in our bodies, particularly in the phospholipids that make up cell membranes. Because of the high concentration of polyunsaturated fatty acids and methylene groups in the double bonds, they are easily oxidised, resulting in the free- radical reaction **(Valko *et al.*, 2004)**. The most significant aldehyde component of lipid peroxidation is malondialdehyde (MDA). It is worth mentioning that MDA was used in medical diagnosis to represent lipid peroxidation rates **(Fukunaka *et al.*, 1995)**. Antioxidants can be present in a number of foods, including vegetables, fruits and grains **(Moon & Shibamoto, 2009)**.

Diverse antioxidants found in seaweeds such as polysaccharides, dietary fibres, minerals, proteins, amino acids, vitamins, polyphenols, and carotenoids have been recorded by several scientists **(Burtin, 2003)**. Seaweeds contain a remarkable range of potential novel antioxidants that help in counteracting the environmental pressures **(Lesser, 2006)**. Natural antioxidants are superior to synthetic antioxidants since they are free of environmental contaminants and perform a wide range of beneficial functions. Such additives are secure to be used in medicines, nutritional supplements, nutraceuticals, including cosmetics to enhance consumer wellbeing, lessen the impact of infectious diseases, besides other wider forms of immune system function. Natural antioxidants are superior to synthetic antioxidants because they do never include chemical pollutants and have a wide range of benefits. These are safe for use as ingredients in medicine, dietary supplements, nutraceuticals, and cosmetics with the objective of improving customer health, reducing the effects of harmful diseases, and other broader aspects of immune system function **(Shahidi, 2009)**. Several countries have reported the antioxidant result of input seaweeds such as Japan **(Matsukawa *et al.*, 1997)**, Indonesia **(Santosa *et al.*, 2004)**, Korea **(Heo *et al.*, 2005)**, India **(Chandini *et al.*, 2008)** and Malaysia **(Matanjun *et al.*, 2008)**. Notably, there is a great deal of interest in monitoring the potential targeted therapies through natural products, with a particular focus on marine entities. Thus, many marine organisms develop biochemical pathways in relation to environmental stresses such as space competition, maintaining unfolded surfaces, avoiding predation, and the ability to reproduce successfully **(Konig *et al.*, 1994)**. Seaweeds are a powerful group of secondary metabolites with a wide range of structures. These bioactive compounds provide resistance to herbivores, fouling species, and pathogens as well as reproduction, UV defence and allopathic agent resistance **(Hay, 1996)**.

Harder (1917) was the first to note seaweed's antimicrobial properties. Bactericidal compounds have been discovered in a variety of algal organisms **(Glombitza, 1979; Banerjee *et al*., 2009)**. Amino acids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, hormones, halogenated ketones including alkanes, cyclic polysulphides, as well as fatty acids are among the compounds present in algae **(Qi *et al*., 2010)**.

Marine food has long been suspected to be high in beneficial lipids; namely, n-3 and n-6 polyunsaturated fatty acids (PUFA). Just a couple of years earlier, eicosapentenoic acid and docosahexenoic acid were considered marine lipids. Several recent studies have comprehensively demonstrated that some lipid compounds, such as carotenoids and sterols, are also essential for human health **(Mori *et al*., 2004)**. In shellfish, algae are considered a significant site of PUFA and carotenoids **(Amornlerdpison *et al*., 2007)**. Brown seaweeds are coloured by marine carotenoids, especially fucoxanthin which is an abundant carotenoid. Several researchers have conclusively reported the antioxidative, anti-cancerous, anti-diabetic, and anti-obesity effects including the unique molecular mechanism responsible for the anti-obesity effects **(Ikeda *et al*., 1998; Yangthong *et al*., 2009; Le *et al*. 2012)**. Fucosterol is a distinctive sterol primarily found in brown seaweeds and is considered to have a lot of health benefits, including hypercholesterolaemic function by reducing cholesterol absorption and antioxidative characteristics **(Lee *et al*., 2004; Nishikawa *et al*., 2012; Nomura *et al*., 2013)**. According to various authors, these are unusual molecules found in a variety of brown seaweed plants **(Terasaki *et al*., 2009)**. Brown seaweeds might be major sources of beneficial lipids for humankind as a result of these useful compounds.

Brown seaweeds might be major sources of beneficial lipids for humankind **(Perez, 2008)**. Microbial infection is a main cause of death in the human species. Furthermore, natural-source complexes are gaining popularity in the drug industry **(Kranz & Dobbelstein, 2012)**.

Cancer is indeed a severe disease that really has afflicted a significant percentage of the world's population throughout history. Although there are various widely used anticancer drugs to cure people with cancer, anticancer agents must act specifically against those tumour cells **(Ji *et al*., 2009)**. The discovery of innovative organic ingredients and additives derived from microorganisms, animals, and plants are extremely and specifically targeting tumour cells, causing no harm for healthy cells **(Canoy *et al*., 2011)**. To increase the positive effect and reduce symptoms of cancer treatment, researchers are looking for organic activities that cause apoptosis in cancer cells which can be used alone or in combination with many other chemotherapeutic drugs **(O'Toole *et al*., 1998)**.

This study was organized to evaluate the antioxidative potential and antimicrobial activity of brown seaweeds; namely, *Sargassum ilicifolium*, *Sargassum tenerrimum*, *Stoechospermum marginatum*, *Padina tetrastromatica*, *Dictyota maxima*, red seaweeds

such as *Gracilaria corticata, Acanthophora spicifera and Batrachospermum* sp., and green seaweeds including *Caulerpa peltata*, *Chaetomorpha crassa,* and *Ulva lactuca.* For the anticancer activity, *Sargassum ilicifolium, Sargassum cinereum,* and *Sargassum tenerrimum* were studied. These seaweeds may be considered by locals as less valuable, but also may present many inconveniences for local fisherman. The seaweed extract can be used as promising anti-oxidative agents, anti-microbial, and anti-cancerous in cosmeceutical or nutraceutical products, which can increase the value of an otherwise worthless weed.

MATERIALS AND METHODS

Sample collection of eleven species of seaweeds viz. *Sargassum ilicifolium, Sargassum tenerrimum, Sargassum cinereum, Stoechospermum marginatum*, *Padina tetrastromatica, Dictyota maxima, Gracilaria corticata, Acanthophora spicifera, Batrachospermum* sp., *Caulerpa peltata*, *Chaetomorpha crassa,* and *Ulva lactuca* were collected from various regions of the Sindhudurg district of Maharashtra in November 2019. Preparation of extract from seaweeds was performed as follows: fresh seaweeds were clean from epiphytes, salt, and sand before being dried at 55°C for 48 h. Dried seaweed was ground into a fine powder. Dried seaweed (100 g) was soaked in 1000 mL of methanol and kept in a shaking incubator at 25°C for 3 days, and the suspension was then filtered through Whatman No. 1 filter paper. The re-extraction process was repeated 3 times. The solvent was pooled, evaporated and lyophilized. The dried extract was dissolved in methanol for different assays **(Santas *et al.*, 2018)**.

Chemicals

All the chemicals and solvents used were of analytical grade. HCL, $FeCL_3$ sodium acetate, ferric chloride, ascorbic acid, galacial acetic acid acetate buffer, 2,4,6- tripyridyl-s-triazine (TPTZ), 2,2- Diphenyl-1- picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), Foetal Bovine Serum (FBS) were obtained from Sigma Chemical Co. Human breast cancer cell-line "MCF-7" was obtained from National Centre for Cell Science, Pune, India.

Antioxidant activity

DPPH (2, 2-Diphenyl-1-picryl-hydrazyl) radical scavenging activity assay was performed with some modifications; 0.5 mL extract was mixed with 3 mL of 25 mM DPPH solution prepared in methanol and incubated for 20 min in dark at room temperature. Absorbance of the resulting solution was measured at 517 nm against a blank (methanol without DPPH). Methanol with DPPH solution was used as the control. Methanol was used as a standard. The per cent of DPPH decolourization of the sample was calculated according to the following equation:

$$\% \ activity = \frac{AC - AS}{AC} x\ 100$$

Where; A_c is the absorbance of control

A_s is the absorbance of sample

FRAP (ferric reducing antioxidant power) assay such as DPPH is also a very commonly used antioxidant assay to analyse the antioxidant capacities of medicinal seaweeds. It's a very simple, rapid, sensitive and inexpensive approach. FRAP method is based on the comparison of the total amount of antioxidant with the reducing capacity of the sample. The reducing capacity of a compound might serve as a significant indicator of its antioxidant capacity. The stock solutions contained 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 50 ml acetate buffer, 5 ml TPTZ solution, and 5 ml $FeCl_3.6H_2O$ solution with the ratio of 10:1:1 and then warmed (incubate) at 37°C before using. Extracts (290 µl) were allowed to react with 10 µl of the FRAP solution for 15 minutes in the dark condition. Absorbance of the coloured product [ferrous tripyridyltriazine complex] was then measured at 595 nm. Results were expressed in mg AAE/g dry mass, compared to those of standard for ascorbic acid. FRAP assay measures the reducing potential of an antioxidant reaction with a ferric tripyridyltriazine (Fe^{3+} – TPTZ) complex and produces a coloured complex of ferrous tripyridyltriazine (Fe^{2+} –TPTZ).

$$\% \ of \ inhibition = \frac{AC - AS}{AC} x\ 100$$

Where; A_c: Absorbance of control

Antimicrobial activity

This study evaluates the antimicrobial activity of ethanolic extract from the seaweeds viz., *Sargassum ilicifolium*, *Sargassum tenerrimum*, *Stoechospermumtum marginatum*, *Padina tetrastromatica*, *Dictyota maxima*, *Gracilaria corticata*, *Acanthophora spicifera*, *Batrachospermum* sp., *Caulerpa peltata*, *Chaetomorpha crassa*, and *Ulva lactuca* from Sindhudurg district along the west coast of Maharashtra. Further, the extracts were screened for their antimicrobial activity against four pathogens; namely, *Klebsiella*, *Bacillus thrungenis*, *Psudeomonus sp.*, and *Streptococ*cus *aureus*. The antimicrobial activity of the seaweeds against pathogens was evaluated according to the methods of the Clinical and Laboratory Standards Institute **(Iqbal *et al.*, 2012)**.

Antimicrobial assay and quantification

In sterile 96 well tissue culture plates containing 200 µl of nutrient broth per well, a 20 µl of fresh bacterial suspension (*Staphylococcus aureus, Bacillus thuringiensis, Psudeomonus* sp., and *Klebsiella*) and 1 µl extract of seaweeds species extract was added in each test wells.

Preparation of bacterial culture

The standard test included 10 ml of nutrient broth, 100 µl bacterial strength and 100µl of standard (streptomycin 1 mg/mL) solution. In addition, every test well received 100 µl of seaweeds extract. After incubation at 37^0C for 24 hours, the content of each well was gently extracted by tapping the plates. The wells were washed with 200 litre of

sterile saline to remove free-floating bacteria. Biofilms produced throughout the plate were stained with 0.1 percent crystal violet and incubated for 20 minutes at room temperature. Excess stain was thoroughly rinsed and washed with deionized water, and plates were fixed with 200 litre of 96% ethanol. Optical densities (OD) of bacteria were measured at 600 nm using UV Spectrophotometer with ELISA microplate reader. The ability of bacteria to shape biofilms was tested with certain modifications following the definitions of **Hecht *et al.* (2007)**.

Anticancer activity

Preparation of extract

Specimens of *Sargassum ilicifolium*, *Sargassum cinereum*, and *Sargassum tenerrimum* were washed using distilled water and were left to dry at room temperature. The dried samples were crushed using a blender. Dried and crushed samples were soaked in ethanol for one day. The filtrate (crude ethanolic fraction) was concentrated using rotary evaporation. Thereafter, the air dried samples were dissolved in dimethyl sulfoxide (DMSO) to a concentration of 4 mg/mL for use in the subsequent assay **(Tung *et al.*, 2015)**.

MTT assay

The MTT assay was used for measuring the cytotoxicity in *Sargassum cinereum*, *Sargassum ilicifolium* and *Sargassum tenerrimum*. Cancerous cells cultured in T-25 flasks were trypsinized and aspirated into a 5 mL centrifuge tube. Cell pellet was obtained by centrifugation at 300 x g. The cell count was adjusted using DMEM HG medium such that 200 µl of suspension contained 10,000 cells. In 96 well plate was incubated at 37^{o}C and 5% CO_2 atmosphere after 24h medium containing of 10% MTT reagent added 0.5 mg/mL, and the plate was incubated at 37^{o}C and 5% CO_2 atmosphere for 3h. Then, the $DMSO_4$ solution was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formosan. The absorbance was measured using a microplate reader at a wavelength of 570 nm.

RESULTS AND DISCUSSION

DPPH radical-scavenging activity

The DPPH assay, which measures the radical-scavenging activity *in vitro* is widely used around the world. The antioxidants throughout the sample scavenge oxidants, as well as the spectrophotometric disappearance of the DPPH radical colour were calculated. This assay evaluates the powerful antioxidants contained in algal extracts for their potential to serve as proton radical scavengers or hydrogen donors **(Qi *et al.*, 2010)**. When methanol extract was measured, the DPPH activity in all the seaweeds in the sample exceeded 44 percent. Similarly, the methanol extracted from *Chaetomorpha linum* contained 45.32 percent DPPH activity in accordance to the findings of **Mori *et al*. (2004)**. When used at a concentration of 10 mg/mL, a commercial antioxidant ascorbic

acid showed a DPPH scavenging activity of 94.75 percent. Notably, a higher DPPH scavenging ability was shown in brown and green seaweeds compared to red seaweeds **(Chakraborty & Paulraj, 2010)**.

The recorded DPPH activity (Table 1) was consistently higher in methanolic extracts of Phaeophyta. The maximum percentage of radical-scavenging activity was observed in the *Padina tetrastromatica* in 6 µl conc. A High DPPH scavenging activity has also been reported in green, brown and red seaweeds **(Wang *et al.*, 2014)**. Brown seaweeds have a high concentration of sulfated polysaccharides, which could be used as usable ingredients for human health. Brown seaweeds have been used to segregate sulfated polysaccharides mostly with diverse therapeutic, nutraceutical and functional food features.

DPPH activity

Extract concentration

Table 1. DPPH activity of common seaweeds

Name of Seaweed	2µl (%)	4 µl (%)	6µl (%)	Average	Mean	Standard deviation
Sargassum ilicifolium	4.90	7.50	20.17	12.53	4.9	10.79
Sargassum tenerrimum	7.61	23.70	11.68	14.33	7.61	8.36
Stoechospermum marginatum	17.60	22.50	3.30	14.46	3.3	9.97
Padina tetrastromatica	19.20	38.00	44.20	33.8	19.2	13.01
Dictyota maxima	0.20	13.80	12.00	8.66	0.2	7.38
Gracilaria corticata	0.08	5.02	23.40	9.5	0.08	12.28
Acanthophora spicifera	1.90	2.00	3.89	2.59	1.9	1.12
***Batrachospermum* sp.**	0.34	0.17	33.4	11.3	0.17	19.13
Caulerpa peltate	3.46	3.7	3.46	3.54	3.46	0.13
Chaetomorpha crassa	6.75	0.675	8.13	5.18	0.675	3.96
Ulva lactuca	0.25	3.59.6	9.6	4.92	0.25	6.61

FRAP assay

The ferric-reducing antioxidant function of different seaweed species is shown in Table (2). The FRAP assay showed a highest antioxidant activity in methanolic extract of the green seaweed *Ulva.* The most FRAP activity was observed in *U. lactuca* (81.80%) followed by *C. peltata* and *Batrachospermum*, while less activity was detected in *S. ilicifolium* (48.70%). The antioxidant activity pattern of methanolic solvents varied due to the presence of various compounds with different species. A high value of astaxanthin

has been recorded in green alga, *Ulva intestinalis* **(Banerjee *et al.*, 2009)**. Chronic consumption of polysaccharides supplied by *Ulva* species, prevents the fall of antioxidant defences and the development of atherosclerosis in hamsters **(Godard *et al.*, 2009)**. The natural Ulvan and its derivatives have been reported to exhibit much higher scavenging activity on superoxide radical compared to vitamin C **(Qi *et al.*, 2010**). Sesquiterpenoids have been isolated from *Ulva fasciata* with free radical scavenging properties **(Chakraborty & Paulraj, 2010)**. Polysaccharides from *U. lactuca* extract with antioxidant effects in experimentally-induced hypercholesterolemic animal model have been reported in the study of **Hassan *et al.* (2011)**.

Table 2. FRAP assay of common Seaweeds

Name of Seaweed (FRAP Assay)	**Mean**	**conc. mg/µl**	**% of Inhibition**
Sargassum ilicifolium	1.1530	6.5325	48.70
Sargassum tenerrimum	0.8155	4.9277	63.72
Stoechospermum marginatum	0.6910	4.3357	69.27
Padina tetrastromatica	0.8035	4.7355	64.25
Dictyota maxima	0.8000	4.8540	64.40
Gracilaria corticate	0.6595	2.0860	70.66
Acanthophora spicifera	0.6635	4.0883	70.48
Batrachospermum sp.	0.6375	3.9680	71.64
Caulerpa peltate	0.6295	4.0432	71.99
Chaetomorpha crassa	0.7855	4.6523	65.05
Ulva lactuca	0.4090	2.9116	81.80
Standard Deviation	0.1822	1.1422	8.1096

Antimicrobial activity

The antimicrobial activity of seaweeds, including *Sargassum ilicifolium*, *Sargassum tenerrimum*, *Stoechospermumtum marginatum*, *Padina tetrastromatica*, *Dictyota maxima*, *Gracilaria corticata*, *Acanthophora spicifera*, *Batrachospermum* sp., *Caulerpa peltata*, *Chaetomorpha crassa*, and *Ulva lactuca* extracts was studied by ethanolic solvents against antimicrobial activity of *Staphylococcus aureus, Bacillus thuringiensis, Pseudomonas* and *Klebsiella* human pathogens. Table (3) demonstrates the in vitro antimicrobial activity of different seaweed extracts against *Staphylococcus aureus, Bacillus thuringiensis, Pseudomonas* and *Klebsiella.* The ethanol extract of *Sargassum tenerrimum* and *Caulerpa peltata* exhibited maximum inhibition on the growth of the tested *Klebsiella* species.

Table 3. Antimicrobial activity

Sr. No.	Name of Seaweed	*Staphylococcus aureus*	*Bacillus thuringiensis*	*Pseudomonas*	*Klebsiella*
1.	*Sargassum ilicifolium*	-	-	-	-
2.	*Sargassum tenerrimum*	-	-	-	+
3.	*Stoechospermum marginatum*	-	-	-	-
4.	*Padina tetrastromatica*	-	-	-	-
5.	*Dictyota maxima*	-	-	-	-
6.	*Gracilaria corticate*	-	-	-	-
7.	*Acanthophora spicifera*	-	-	-	-
8.	*Batrachospermum sp.*	-	--	-	-
9.	*Caulerpa peltata*	-	-	-	+
10.	*Chaetomorpha crassa*	-	-	-	-
11.	*Ulva lactuca*	-	-	-	-

Anticancer activity

Cytotoxic activity

In this study, Tables (4, 5& 6) show the anti-cancer activity of *Sargassum* spp. extract tested against breast cancer cells MCF7 by using MTT assay.

Effects in vitro cytotoxic of alcoholic extract of *Sargassum* spp. were screened against MCF-7 human breast cancer cell line, and the viability of tumour cell was confirmed by MTT assay. The *in vitro* cytotoxic of alcoholic extract of *S. cinereum*, *S. tenerrimum*, *S.ilicifolium* was measured using an MTT assay. The 25 to 400 µl/ml concentration for 24 hours were found to be cytotoxic, and the cell percentages of viability of *S. cinereum* - 62.18, 18.83, 1.73, 2.07, 4.15, for the *S. tenerrimum* - 57.86, 26.17, 1.73, 2.68, 3.89 and *S.ilicifolium* - 76.94, 35.41, 1.64, 2.59, 5.09 at concentration 25, 50, 100, 200, 400 µl/ml, respectively.

Table 4. Effect of extract of *Sargassum cinereum* on Breast cancer (MCF-7 cell line)

MCF-7 cell line	Test concentrations (µL/ml)- S. *cinereum*					
	Untreated	25	50	100	200	400
Reading 1	0.616	0.414	0.142	0.011	0.015	0.025
Reading 2	0.545	0.309	0.079	0.012	0.012	0.026
Mean OD	0.581	0.362	0.111	0.012	0.014	0.026
Mean Blank	0.5790	0.3600	0.1090	0.0100	0.0120	0.0240
Standard deviation	0.0502	0.0742	0.0445	0.0007	0.0021	0.0007
Standard error	0.0355	0.0525	0.0315	0.0005	0.0015	0.0005
% Standard error	6.1313	9.0674	5.4404	0.0864	0.2591	0.0864
% Viability	100	62.18	18.83	1.73	2.07	4.15

IC50= 18.62µL/ml

Table 5. Effect of extract of *S. tenerrimum* on Breast cancer (MCF-7 cell line)

MCF-7 cell line	Test concentrations (µL/ml)- S. *tenerrimum*					
	Untreated	25	50	100	200	400
Reading 1	0.616	0.377	0.164	0.009	0.015	0.024
Reading 2	0.545	0.296	0.142	0.014	0.019	0.024
Mean OD	0.581	0.337	0.153	0.012	0.017	0.024
Mean OD Blank	0.5790	0.3350	0.1515	0.0100	0.0155	0.0225
Standard deviation	0.0502	0.0573	0.0156	0.0035	0.0028	0.0000
Standard error	0.0355	0.0405	0.0110	0.0025	0.0020	0.0000
% Standard error	6.1313	6.9948	1.8998	0.4318	0.3454	0.0000
% Viability	100	57.86	26.17	1.73	2.68	3.89

IC50= 18.96µL/ml

Anticancer activity polysaccharides fraction of *Sarggasum wightii* reduced the growth of MCF-7 cells significantly. The inhibition of polysaccharides fraction against MCF-7 cells was 69% at the concentration of 500 µg/ml, and IC50 was calculated as 350 µg/ml **(Vaikundamoorthy *et al.*, 2018)**. *S. angustifolium* extracted with hexane, and the dichloromethane to inhibition of MCF-7 cell were the IC50 value 200 µg/ml and 250 µg/ml **(Vaseghi *et al.*, 2020)**.

Concentration of 25 µl/ml and lower concentration of *S. cinereum, S. tenerrimum*, and *S.ilicifolium* reduces the viability of MCF-7 cells and IC50 value of *S. cinereum* -

18.62 (Table 4), *S. tenerrimum* - 18.96 (Table. 5) and *S.ilicifolium* - 36.48µL/ml (Table 6).

Table 6. Effect of extract of *S.ilicifolium* on Breast cancer (MCF-7 cell line)

MCF-7 cell line	Test concentrations (µL/ml) – *S.ilicifolium*					
	Untreated	25	50	100	200	400
Reading 1	0.616	0.473	0.245	0.009	0.015	0.022
Reading 2	0.545	0.421	0.168	0.013	0.018	0.040
Mean OD	0.581	0.447	0.207	0.011	0.017	0.031
Mean OD-Mean Blank	0.5790	0.4455	0.2050	0.0095	0.0150	0.0295
Standard deviation	0.0502	0.0368	0.0544	0.0028	0.0021	0.0127
Standard error	0.0355	0.0260	0.0385	0.0020	0.0015	0.0090
% Standard error	6.1313	4.4905	6.6494	0.3454	0.2591	1.5544
% Viability	100	76.94	35.41	1.64	2.59	5.09

IC50= 36.48µL/ml

CONCLUSION

Green, red, and brown seaweeds can be used to improve immunity and inhibit oxidative stress in humans according to the afore- mentioned antioxidant and antimicrobial reports. The therapeutic effect of seaweeds has been found to be promising. In this context, seaweeds may be an excellent nutritional supplement and a general mineral deficiency treatment. Seaweeds, in the current research, proved their safety, thus they may be used as a safe tea or as an antioxidative agent.

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