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EVALUATION OF *IN VITRO* ANTICANCER AND ANTIOXIDANT ACTIVITY OF MARINE MICRO ALGAL EXTRACTS

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ABSTRACT

The present study was aimed to evaluate the anticancer and antioxidant activity of the five algal samples named as S–1 to S–5 isolated from the seawater collection of Muttom coastal region of Kanyakumari District. Mass production of micro algae was done by using sea water containing Walne's medium and each cultivated algae was isolated by centrifugation and extracted with different solvents viz., ethyl acetate, methanol and water. The extracts obtained were evaluated for *in vitro* anticancer and antioxidant activity. Anticancer activity was evaluated by MTT assay using the cell lines MCF7 and SKMEL. In this evaluation, extracts of all the algal samples showed anticancer activity against the selected cell lines, however, the methanol extract of S–2 sample showed significant activity comparing with other samples. Antioxidant activity was evaluated by DPPH, reducing power assay and superoxide radical scavenging assay methods. In this evaluation also, the methanol extract of S–2 sample showed a significant antioxidant activity in all the tested methods. These results are useful for further investigation in the future.

KEYWORDS: Marine microalgae, extraction, anticancer activity, antioxidant activity.

INTRODUCTION

Natural products are in usage for therapeutic purpose throughout the world since ancient time. Terrestrial plants are a major source of traditional medicinal system. Nowadays about 25% of modern drugs are derived from higher plant origin and about 12% are microbial origin and over 50% of them are derived from natural sources either directly or indirectly or produced synthetically using natural products as templates.[1-3] In the last few decades, bacteria and fungi of terrestrial origin are well screened and as a result, several bio-active molecules are isolated and now in usage as important medicines.[2]

In the natural world, ocean is a distinctive environment and habitat for numerous organisms, and also an enormous source for food, minerals, energy and pharmaceuticals.[2] The marine environment has vast bio-diversity which is unparalleled to terrestrial environment because of its great differences in thermal, pressure and nutrient range and the presence of different photic and non-photic zones.[1,4] The search for newer biomedical molecules resulted in the isolation of thousands of metabolites from marine origin many of which revealed therapeutic properties including antibiotic, antifungal, antiviral, cytotoxic, neurotoxic, etc.[5,6] Microalgae, the photosynthetic eukaryotes that forms a major part of freshwater and marine phytoplankton[2] has gains more attention in life sciences because of their variety of phytomolecules with different types of chemical structures and biological activities.[2,6,7] With this view, the marine microalgae was selected for our research.

In our previous study[8] totally 10 algal samples were isolated from the sea water samples collected from the coastal region of Kanyakumari District of Tamil Nadu such as Colachel, Muttom and Kadiapattinam. Based on the growth pattern showed and the yield obtained, five samples named as S-1 to S-5 isolated from the Muttom seawater collection was evaluated for the antimicrobial activity. The present study was aimed to evaluate the antioxidant and anticancer activity *in vitro* of the algal extracts of Muttom seawater collection.

MATERIALS AND METHODS

Micro algal extract preparation

In our previous study, marine water samples were collected from the different coastal locations such as Colachel, Kadiapattinam and Muttom of Kanyakumari District, South India. Mass production of micro algae was done by using sea water containing appropriate quantity of Walne's medium and each cultivated algae was isolated by centrifugation. Dried algal cell (0.4gm) were mixed with 20ml of different solvents (Ethyl acetate, Methanol and Water), shaken well and each mixture was subjected to sonication for 15min. and centrifugation at 3000rpm for 10min. The supernatants were collected and stored.[8] The algal extracts of Muttom seawater collection was evaluated for antioxidant and anticancer activity.

***In vitro* anti cancer activity**

Sub culturing and maintenance of cell line

In vitro anti cancer evaluation by MTT assay was designed with the reference of standard procedure.[2] The cell lines MCF7 and SKMEL procured from NCCS, Pune, India were used for the study. They were maintained in 10% heat inactivated FBS (Foetal Bovine Serum) in CO_2 atmosphere. The cells were trypsinised (500 ml of 0.025% Trypsin in Phosphate Buffered Saline (PBS)/Ethylene Diamine Tetra Acetic acid EDTA solution) for 2min. and sub cultured in micro culture plates and used for further studies. Anticancer activity of selected micro algal extracts (500µg/ml) was determined by standard MTT assay on the cultured cell lines.

MTT assay

The cell culture suspension was washed with Phosphate Buffered Saline (1**x**PBS) and then added MTT [3-(4,5–Dimethylthiazole–2yl)-2,5-diphenyltetrazolium bromide] solution (200ml) to the culture flask (MTT 5mg/ml dissolved in PBS, filtered (0.2µm filter) before use and incubated at 37°C for 3h. All MTT solution was removed washed with 1xPBS and 300µl DMSO (Dimethylsulfoxide) was added to each culture flask and incubated at room temperature for 30min. until all cells get lysed and homogenous color was obtained. Then, centrifuged and precipitate the cell debris which was dissolved by using DMSO. The OD was measured at 540nm using DMSO blank, and the percentage viability was calculated by

$$\text{Cell viability (\%)} = \frac{\text{Mean OD}}{\text{Control OD}} \text{ x } 100$$

***In vitro* antioxidant activity**

Antioxidant activity was evaluated by different approaches such as DPPH, reducing power assay, superoxide radical scavenging assay methods.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

Evaluation of antioxidant activity by DPPH assay was done in reference with the previous literature.[9-11] In this evaluation, 1ml of test extracts in different concentration such as 100, 200, 400 and 800µg/ml was mixed with 1ml of 0.1mM methanolic solution of DPPH and incubated at room temperature in dark condition for 30min. After incubation, the absorbance of reaction mixtures was measured spectrophotometrically at 517nm. Methanol was used as blank; DPPH in methanol and the ascorbic acid was used as control and standard control respectively. The percentage inhibition of DPPH by the test extracts was determined by

$$\text{Percentage inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

Reducing power assay

This method of evaluation of antioxidant activity was done with the reference of standard procedure.[11,12] In this method, 500µl of test extracts in different concentration such as 100, 200, 400 and 800µg/ml was mixed with 1.5ml of 0.2M sodium phosphate buffer in 6.6 pH and 1.5ml of 1% potassium ferricyanide and kept for incubation at 50°C for 20min. 5ml of 10% trichloroacetic acid was added to the incubated mixture and subjected to centrifugation at 3000rpm for 6min at 4°C. To 1.5ml of the upper layer of centrifuged collected was mixed with equal volume of distilled water and 300µl of 0.1% freshly prepared ferric chloride solution and kept for 10min. Then, the absorbance of the mixture was measured spectrophotometrically at 700nm. Ascorbic acid was used as standard control.

Superoxide radical scavenging assay

This evaluation was designed with the reference of previous literature.[11,13] The reaction mixture in 3ml contains test extracts in different concentration such as 100, 200, 400 and 800µg/ml, 0.1ml of 1.5mM Nitro blue tetrazolium solution, 0.2ml of 0.1M EDTA, 0.05ml of 0.12mM riboflavin and 2.55ml of 0.067M phosphate buffer. DMSO was used in control tubes instead of sample. Ascorbic acid was used as the standard control. The reaction mixture was kept for 30min. in front of fluorescent light of 34W and then the absorbance was measured spectrophotometrically at 560nm. All the tests were done in triplicate and the results were averaged. The percentage inhibition superoxide radical was calculated by

$$\text{\% inhibition} = \frac{\text{Control Abs.} - \text{Sample Abs.}}{\text{Control Abs.}} \text{ X } 100$$

RESULTS AND DISCUSSION

In the present study, five algal isolates named as S–1 to S–5 from the Muttom seawater collection[8] was selected for the evaluation of anticancer and antioxidant activity. Anticancer activity of the algal extracts was evaluated *in vitro* by MTT assay against MCF7 and SKMEL cell line. From the results it was found that extracts of all the algal samples showed anticancer activity against the selected cell lines, however, the methanol extract of S–2 sample showed significant activity comparing with other samples (Table 1 & 2).

Table 1: MTT assay on MCF7 cell line.

Isolated algae	Extracts	OD	Cell viability (%)
S–1	Control	0.732	100
	Ethyl acetate	0.405	55.3
	Methanol	0.391	53.4
	Water	0.407	55.6
S–2	Control	0.732	100

	Ethyl acetate	0.309	42.2
	Methanol	0.205	28.0
	Water	0.425	58.0
S–3	Control	0.732	100
	Ethyl acetate	0.390	53.2
	Methanol	0.350	47.8
	Water	0.410	56.0
S–4	Control	0.732	100
	Ethyl acetate	0.389	53.1
	Methanol	0.327	44.6
	Water	0.413	56.4
S–5	Control	0.732	100
	Ethyl acetate	0.403	55.0
	Methanol	0.310	42.3
	Water	0.437	59.6

Table 2: MTT assay on SKMEL cell line.

Isolated algae	Extracts	OD	Cell viability (%)
S–1	Control	0.763	100
	Ethyl acetate	0.411	53.8
	Methanol	0.368	48.2
	Water	0.425	55.7
S–2	Control	0.763	100
	Ethyl acetate	0.342	44.8
	Methanol	0.234	30.6
	Water	0.429	56.2
S–3	Control	0.763	100
	Ethyl acetate	0.397	52.0
	Methanol	0.330	43.2
	Water	0.415	54.3
S–4	Control	0.763	100
	Ethyl acetate	0.402	52.6
	Methanol	0.306	40.1
	Water	0.397	52.3
S–5	Control	0.763	100
	Ethyl acetate	0.408	53.4
	Methanol	0.360	47.1
	Water	0.416	54.5

Regarding with antioxidant activity evaluation, from the results, it was found that the antioxidant activity was increases with the increase of concentration of test extracts. All the test extracts revealed a maximum activity in the highest concentration (800µg/ml). However, the methanol extract of S–2 sample showed a significantly higher activity comparing with other sample extracts. The methanol extract of S–2 sample showed a percentage inhibition of 89.13±2.10 in 800µg/ml concentration. Similar concentration of standard agent, ascorbic acid employed for the comparative evaluation exhibited a percentage inhibition of 75.53±2.31. These results clearly indicated the antioxidant potential of the methanol extract of the sample S–2 (Table 3).

Table 3: Antioxidant activity of algal extracts in DPPH assay.

		Percentage inhibition			
Algal sample	**Con. (µg/ml)**	**Extracts**			
		Ethyl acetate	**Methanol**	**Water**	**Std.**
S–1	100	22.53±1.36	41.52±1.27	10.24±0.52	45.35±0.17
	200	34.46±1.25	55.35±1.44	15.36±0.21	57.42±1.33
	400	46.31±1.50	67.50±1.25	24.45±0.35	63.21±1.22
	800	68.27±1.31	81.37±1.32	30.31±0.55	75.53±2.31
S–2	100	26.68±1.32	48.43±1.47	10.69±0.32	45.35±0.17
	200	33.51±1.75	61.49±1.68	16.47±0.43	57.42±1.33
	400	48.43±1.77	73.16±2.15	27.15±0.56	63.21±1.22

	800	69.58±1.24	89.13±2.10	33.47±0.41	75.53±2.31
S–3	100	31.48±1.22	44.47±1.42	10.29±0.12	45.35±0.17
	200	36.52±1.35	57.43±1.58	13.42±0.48	57.42±1.33
	400	45.49±1.27	69.14±2.35	27.15±0.56	63.21±1.22
	800	65.54±1.26	77.15±2.12	31.37±0.41	75.53±2.31
S–4	100	29.28±1.72	45.33±1.27	11.63±0.22	45.35±0.17
	200	31.56±1.45	56.19±1.38	15.17±0.53	57.42±1.33
	400	40.45±1.37	65.13±2.13	21.13±0.53	63.21±1.22
	800	59.53±1.26	73.15±2.13	29.33±0.45	75.53±2.31
S–5	100	23.18±1.22	39.13±1.37	10.59±0.62	45.35±0.17
	200	31.55±1.35	54.19±1.58	13.47±0.83	57.42±1.33
	400	44.23±1.67	67.13±1.15	23.12±0.55	63.21±1.22
	800	63.18±1.23	73.12±1.10	29.67±0.31	75.53±2.31

The next method, reducing power assay method employed for the evaluation of antioxidant activity also showed a concentration dependent rise of antioxidant activity in all the tested extracts of samples. In this method also, the methanol extract of S–2 sample proved its antioxidant potential with the percentage inhibition of 92.38±1.47. In this method, the standard agent ascorbic acid revealed a percentage inhibition of 57.41±1.45 (Table 4).

Table 4: Antioxidant activity of algal extracts in reducing power assay.

		Percentage inhibition			
Algal sample	**Con. (µg/ml)**	**Extracts**			
		Ethyl acetate	**Methanol**	**Water**	**Std.**
S–1	100	31.43±1.23	45.47±1.23	13.10±0.34	25.56±0.47
	200	42.65±2.09	51.41±1.63	21.36±0.67	33.42±0.47
	400	53.45±1.34	59.11±1.35	32.56±0.45	46.37±1.05
	800	69.23±1.42	71.21±1.21	41.31±0.55	57.41±1.45
S–2	100	35.25±1.50	54.31±1.26	13.26±0.15	25.56±0.47
	200	43.43±2.31	67.37±1.23	21.31±0.47	33.42±0.47
	400	55.32±1.25	73.42±1.41	33.46±1.53	46.37±1.05
	800	66.40±1.20	92.38±1.47	44.25±1.39	57.41±1.45
S–3	100	33.53±1.63	41.45±1.63	15.12±0.54	25.56±0.47
	200	45.25±2.13	53.42±1.33	23.32±0.57	33.42±0.47
	400	51.41±1.44	61.15±1.45	35.55±0.25	46.37±1.05
	800	65.13±1.52	73.25±1.41	43.21±0.35	57.41±1.45
S–4	100	29.45±1.33	47.37±1.33	11.22±0.54	25.56±0.47
	200	37.45±2.10	55.11±1.23	23.56±0.47	33.42±0.47
	400	49.43±1.24	63.13±1.55	35.16±0.55	46.37±1.05
	800	57.25±1.22	75.31±1.31	43.41±0.25	57.41±1.45
S–5	100	35.41±1.25	42.37±1.33	10.23±0.54	25.56±0.47
	200	49.15±2.10	53.45±1.53	25.16±0.37	33.42±0.47
	400	57.25±1.44	62.19±1.25	37.46±0.25	46.37±1.05
	800	71.22±1.52	77.51±1.43	45.11±0.45	57.41±1.45

The superoxide radical scavenging assay, another one method employed for assessing the antioxidant ability of test extracts also revealed a similar kind of results. In this method also, comparing with other tested samples and the standard agent (ascorbic acid), the methanol extract of S–2 sample revealed a maximum activity with a percentage inhibition of 77.35±0.41 in 800µg/ml (Table 5).

Table 5: Antioxidant activity of algal extracts in superoxide radical scavenging assay.

		Percentage inhibition			
Algal sample	**Con. (µg/ml)**	**Extracts**			
		Ethyl acetate	**Methanol**	**Water**	**Std.**
S–1	100	10.48±0.25	21.11±0.50	09.31±0.48	61.45±0.23
	200	15.18±0.10	36.30±0.20	13.28±0.37	75.32±0.43
	400	26.48±0.76	45.34±0.25	19.40±0.59	87.53±0.15
	800	39.55±0.10	65.40±0.52	21.15±0.59	95.21±0.36

S–2	100	13.27±0.27	25.37±0.21	09.21±0.78	61.45±0.23
	200	21.41±0.32	39.50±0.30	11.58±0.47	75.32±0.43
	400	29.36±0.43	55.37±0.26	13.20±0.89	87.53±0.15
	800	45.42±0.10	77.35±0.41	19.26±0.69	95.21±0.36
S–3	100	15.38±0.35	24.23±0.40	11.21±0.38	61.45±0.23
	200	19.13±0.20	35.33±0.50	19.18±0.57	75.32±0.43
	400	28.38±0.56	47.14±0.15	23.10±0.39	87.53±0.15
	800	39.25±0.09	55.56±0.32	29.25±0.49	95.21±0.36
S–4	100	13.35±0.31	19.31±0.30	13.41±0.36	61.45±0.23
	200	21.19±0.23	27.20±0.13	19.36±0.27	75.32±0.43
	400	36.38±0.26	34.54±0.35	22.45±0.39	87.53±0.15
	800	47.45±0.30	45.31±0.42	29.25±0.39	95.21±0.36
S–5	100	11.35±0.35	23.31±0.40	10.25±0.58	61.45±0.23
	200	19.28±0.30	32.40±0.30	15.18±0.47	75.32±0.43
	400	26.18±0.36	44.21±0.27	21.32±0.47	87.53±0.15
	800	37.25±0.21	59.10±0.32	27.51±0.36	95.21±0.36

In the evaluation of antioxidant activity a concentration dependent increase of activity was found in the results. Previous reports on terrestrial plant sources[14,15] revealed a similar type of results. In the present study it was found that the methanol extract showed a very significant antioxidant activity comparing with other tested extracts which is in accordance with a previous report.[16] Generally, microalgae produce a wide range of antioxidant compounds such as carotenoids, phenolic compounds, polysaccharides and long chain polyunsaturated fatty acids.[16] Presence of these bioactive molecules in the tested sample may responsible for the significant antioxidant activity.

CONCLUSION

In the present study, five algal samples named as S–1 to S–5 isolated from the seawater collection of Muttom coastal region of Kanyakumari District was mass cultured, extracted and the extracts obtained were evaluated for anticancer and antioxidant activity *in vitro*. Anticancer activity was evaluated by MTT assay using the cell lines MCF7 and SKMEL. Antioxidant activity was evaluated by DPPH, reducing power assay and superoxide radical scavenging assay methods. In the anticancer evaluation, all the algal samples showed anticancer activity against the selected cell lines, however, the methanol extract of S–2 sample showed significant activity comparing with other samples. In the antioxidant evaluation also, the methanol extract of S–2 sample showed a significant antioxidant activity in all the tested methods. Our further studies directed toward the phytochemical screening of this extract and the identification algal species and screening for other biological activities may give significant results.

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