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Research Article

Invitro Antiproliferative Effect Of Marine Macroalgae On Different Human Cancer Cell Lines

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Abstract

Natural compounds from marine ecosystem constitute the most promising source of novel bioactive compounds with potential function in human therapeutics and cancer treatment. Most importantly, marine algae were proved to be an important source of bioactive agents. The present study was aimed to evaluate the *in vitro* antiproliferative/anticancer properties of selected marine macroalgae extracts (n=34), derived from different species of brown and red marine macroalgae on lung cancer (NCI-H1975), prostate cancer (LnCaP), breast cancer (MCF7) and epidermoid cancer (A431) cell lines. The algae extracts were assayed for their anticancer properties on 4 human cancer cell lines using XTT cell proliferation assay. On screening of 34 algae extracts, the ethanol and water extract of brown algae *Turbinaria ornata* (S4 and S3) exhibited remarkably high anticancer activity on NCI-H1975 (lung) and LnCaP (prostate) cancer cells. Hence, the bioactive components from marine macroalgae might be used as potential therapeutic candidate against cancer.

Keywords: Macroalgae, Cancer, XTT assay, Seaweed. *Turbinaria ornate*, Cell lines

Introduction

Cancer is the second leading cause of death and complex genetic disease, which is characterized by the uncontrolled proliferation and spread of abnormal cells and is the most progressive disease posing a threat of mortality worldwide. As per statistical data, 8.8 million deaths were enrolled by different cancers world wide in 2015 [(1)]. It is estimated that approximately 26 million new cases will be enrolled by 2030, and the quantum would increase in future around the globe [(2)].

Different treatment methods like chemotherapy, radiation, surgery and combinations of these are standard treatment strategies for most of the cancers. Majority of the anticancer drugs, which are used in chemotherapy are hazardous to the normal cells, which causes severe side effects. The invention of new drugs with low or devoid of side effects on patients would become a milestone in immunopharmacology [(3, 4)]. Seaweeds are a group of non-flowering marine plants commonly known as macroalgae and are one of the potential sources of biologically active compounds due to their enormous biodiversity[(5)]. Since ages some of the seaweeds have been used as Asian traditional medicine. From the past few years, isolation of antiproliferative substances from marine organisms has been reported[(6)]. Most of the bioactive compounds were isolated from marine algae, such as carotenoids, proteins, polysaccharides, essential fatty acids, antioxidants, vitamins and minerals [(7)].

Till today, more than 2,400 substances were reported from marine flora of different populations [(8)]. Studies proved that seaweeds contain antitumor, anti-oxidant, anti-viral, anti-bacterial and anti-fungal activities [(9, 10)]. More than 10 new anticancer agents were derived from marine sources

are used in clinical trials including aplidine, bryostatinn, ecteinascidin, kahalalide-F, and their derivatives [11]. Further research is underway to identify the natural substance with less or devoid of side effects [12].

The current study was aimed to evaluate the antiproliferative properties of water, ethanol and methanol extracts (n=34) derived from different brown and red marine macroalgae on 4 different cancer cell types i.e., NCI-H1975 (lung cancer), MCF-7 (breast cancer), LnCaP (prostate cancer) and A431 (epidermoid cancer) initially at 500, 50 and 5 µg concentration, and also to determine the IC_{50} value of the selected algae extracts on 4 different cancer cell lines using XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) cell proliferation assay [13,14].

Materials And Methods

Seaweed raw material collection and processing

The details of brown and red marine macroalgae used in this study (n=34) was represented in Table 1. The identities of the seaweeds considered in this study were ascertained with the sample specimens maintained in the Marine Biodiversity Museum of CMFRI. The samples were collected from the Gulf of Mannar, India during the months of August 2011 to April 2012. The samples (2 kg) were washed in running water and shade dried before being pulverized to a minimum particle size. The powder form of marine macroalgae samples (1kg) were extracted in n-hexane (37°C for 24 hours) and the pigments were separated. The filtrate was collected by using Whatman No.1 filter paper and successively extracted with alcohols (methanol and ethanol) at 60°C. Thereafter the filtrate was concentrated at 50°C in a rotary vacuum evaporator (Heidolph, Germany) to afford dark brown viscous mass of methanol and ethanol extracts respectively.

The water extracts of the seaweed were prepared by extracting the dried seaweed powder (500g) with hot water (80°C) for 4 hours. The contents were cooled and centrifuged at 8500 rpm (4°C) for 15 minutes and freeze-dried to get the crude water extract.

Cell lines and culture condition

The NCI-H-1975 and LnCaP cells were cultured in RPMI-1640 medium, whereas the A431 and MCF7 cells were cultured in DMEM medium. All cell lines are maintained at optimum temperature 37^0C with 5% CO_2.

Experimental design

The cell based treatment was initiated with marine macroalgae extracts (n=34) first at 500, 50 and 5µg on 4 different cancer cell lines and followed by the determination of IC_{50} of the selected 4 active algae extracts (S4, S3, H2 and A1) on four different cancer cell lines. The following cancer cells [NCI-H-1975 (1000), A431 (1000), MCF7 (1500) and LnCaP (2000) cells/ well] were seeded in 96 well cell culture plate with growth medium. The 34 extracts derived from the marine macroalgae (Table-1) were serially diluted in culture medium. The cells were treated with algal extracts at 500, 50 and 5µg concentration in triplicate wells per concentration and incubated at 37^oC for 72 hours.

After incubation, 100µL of XTT solution (1mg/ml) was added in culture media and the plates were incubated at 37°C for calorimetric reaction. The absorbance was recorded at 465 nm. Based on the data from 3 point screening, 4 potent extracts were selected and determined the antiproliferative activity at 8 concentrations from 100µg to 0.05µg (half log serial dilution) on 4 different cancer cell lines (NCI-H-1975, A431, MCF7 and LnCaP) for 72 hours using XTT assay.

Data analysis

Absorbance values were normalized to the blank (read out from media). Mean and standard deviations were calculated based on triplicate data points per sample. The values for the various samples were calculated as a percentage of the control treated cells in growth media. These values reflected the percentage of proliferating cells in each sample relative to the positive control. The values obtained were subtracted from 100 to give percent inhibition in proliferation. The data points were entered in the GraphPad Prism software to determine the IC_{50}. The curves were fitted using a nonlinear regression with a sigmoidal dose response.

Table.1: Illustrates the details of various brown and red algae extracts.

Solvent (methanol, ethanol) and aqueous extracts of Brown seaweeds			
S.No	**Sample code**	**Genus name**	**Extract type**
1	A1	*Sargassum wightii*	Aqueous
2	A3	*Sargassum myriocystum*	Aqueous
3	AE3	*Turbunaria conoides*	Ethanolic
4	EM9	*Padina tetrastomatica*	Aqueous
5	G1	*Padina gymnospora*	Aqueous
6	H1	*Hydroclathrus tenuis*	Aqueous
7	H2	*Hydroclathrus tenuis*	Methanolic
8	PS1	*Turbinaria conoides*	Aqueous
9	S1	*Turbinaria ornate*	Ethanolic
10	S2	*Sargassam myriocystum*	Methanolic
11	S3	*Turbinaria ornate*	Aqueous
12	S4	*Turbinaria ornate*	Ethanolic
13	OS1	*Turbinaria conoides*	Methanolic
14	OS2	*Turbinaria conoides*	Ethanolic
15	S7	*Sargassum wightii*	Ethanolic
16	S15	*Sargassum myriocystum*	Aqueous
17	SF	*Sargassum myriocystum*	Ethanolic
18	SS1	*Sargassum wightii*	Methanolic
Solvent (methanol, ethanol) and aqueous extracts of Red seaweeds			
19	EM1	*Hypnea valentiae*	Methanolic
20	EM2	*Laurentia papilosa*	Aqueous
21	EM3	*Jania rubens*	Aqueous
22	F1	*Gracilaria opuntia*	Aqueous
23	FS1	*Kappaphycus alvarezii*	Aqueous
24	FS2	*Hypnea musciformis*	Methanolic
25	FS3	*Gracilaria opuntia*	Methanolic
26	FS4	*Gracilaria opuntia*	Aqueous
27	FS5	*Laurencia papilosa*	Ethanolic
28	K1	*Kappaphycus alvarezii*	Methanolic
29	MP1	*Jania rubens*	Methanolic
30	MV1	*Laurencia papilosa*	Methanolic
31	FM1	*Kappaphycus alvarezii*	Aqueous
32	FM2	*Gracilaria opuntia*	Ethanolic
33	FM3	*Kappaphycus alvarezii*	Ethanolic
34	FM4	*Gracilaria opuntia*	Aqueous

Results

In the present investigation, a total of thirty four algal extracts (Table-1), were selected for screening the antiproliferative/anticancer effect on 4 cancer cell lines (H1975, A431, MCF7 and LnCaP) for 72 hours at 3 concentrations (500, 50 and 5µg). The present study showed the antiproliferative activity of 4 marine macroalgae extracts on different cancer cell lines as follows.

Antiproliferative effect of 34 algae extracts on NCI-H-1975 cells

The antiproliferative effect of 34 algal extracts (brown and red) were determined on NCI-H-1975 cells (Fig.1 A and B). The ethanol extract of brown seaweed *Turbinaria ornata* (S4, 100% at 500µg), the water extract of brown seaweed *Turbinaria ornata*

(S3, 100% at 500µg), methanol extract of *Hydroclathrus tenuis* (H2, 99% at 500µg) and water extract of *Sargassum wightii* (A1, 83% at 500µg) showed inhibition in H-1975 cell proliferation.

Antiproliferative effect of 34 algae extracts on A431 cells

The antiproliferative effect of 34 algae extracts (brown and red) were determined on A431cells (Fig.2 A and B). The ethanol extract of brown seaweed *Turbinaria ornata* (S4, 92% at 500µg), the water extract of brown seaweed *Turbinaria ornata* (S3, 95% at 500µg), methanol extract of Hydroclathrus tenuis (H2, 78% at 500µg) and water extract of Sargassum wightii (A1, 62% at 500µg) showed inhibition in A431 cell proliferation.

Antiproliferative effect of 34 algae extracts on MCF7 cells

The antiproliferative effect of 34 algal extracts (brown and red) were determined on MCF7cells (Fig.3 A and B). The ethanol extract of brown seaweed *Turbinaria ornata* (S4, 14% at 500µg), the water extract of brown seaweed *Turbinaria ornata* (S3, 53% at 500µg), methanol extract of Hydroclathrus tenuis (H2, 47% at 500µg) and water extract of Sargassum wightii (A1, 15% at 500µg) did not show significant inhibition in MCF7 cell proliferation.

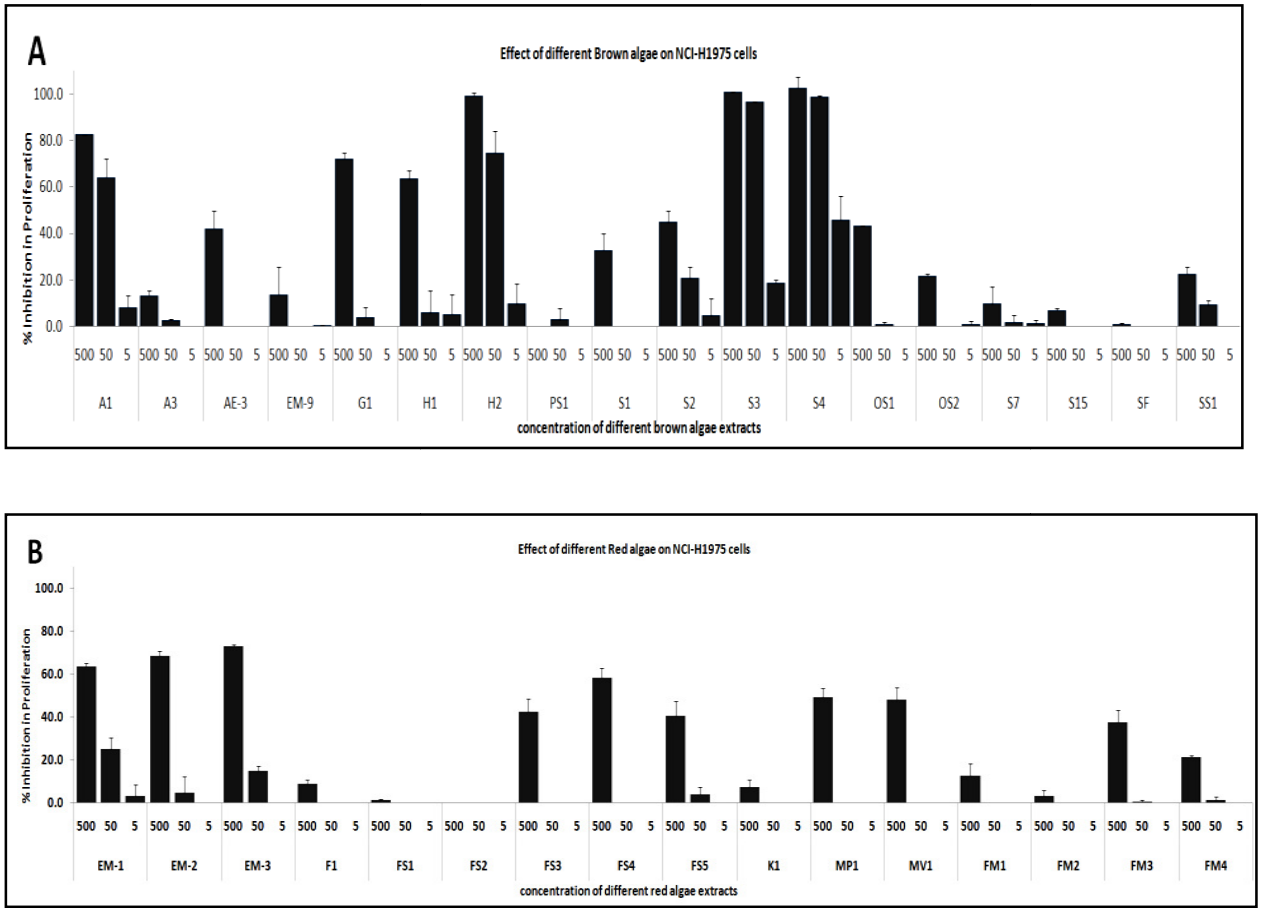

Fig-1: Effect of macroalgae extracts (n=34) A. Brown algae B. Red algae on NCI-H-1975 (Lung cancer) cells

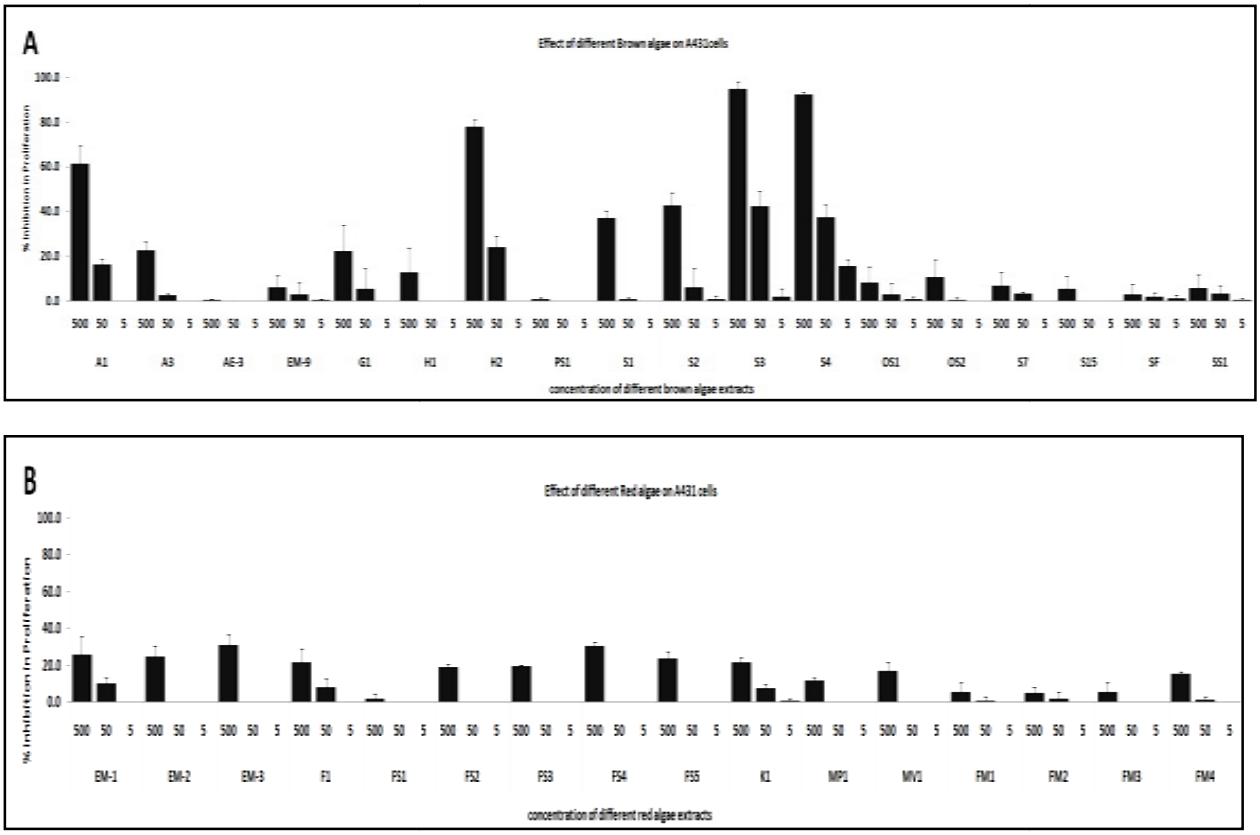

Fig-2: Effect of macroalgae extracts (n=34) A. Brown algae B. Red algae on A431 (Epidermoid cancer) cells

Antiproliferative effect of 34 algae extracts on LnCaP cells

The antiproliferative effect of 34 algal extracts (brown and red) were determined on LnCaP cells (Fig.4 A and B). The ethanol extract of brown seaweed *Turbinaria ornata* (S4, 90% at 500µg), the water extract of brown seaweed *Turbinaria ornata* (S3, 93% at 500µg), methanol extract of Hydroclathrus tenuis (H2, 56% at 500µg) and water extract of Sargassum wightii (A1, 38% at 500µg) showed inhibition in LnCaP cell proliferation.

The IC_{50} value of 4 potent algae extracts on 4 different cancer cells

Based on the above data sets, 4 active marine macro algal extracts (S4, S3, H2 and A1) were selected for full dose response (100.00, 33.33, 11.11, 3.70, 1.23, 0.41, 0.14 and 0.05µg) screening on 4 different cancer cell lines (NCI-H1975, A431, MCF7 and LnCaP) for 72 hours.

The selected 4 algal extracts (S4, S3, H2 and A1) showed antiproliferative activity on NCI-H-1975 cells (Fig-5a). The ethanol extract of brown seaweed *Turbinaria ornata* (S4, IC_{50}-5.97µg), the water extract of brown seaweed *Turbinaria ornata* (S3, IC_{50}-12.56µg), methanol extract of *Hydroclathrus tenuis* (H2, IC_{50}-36.84µg) and water extract of *Sargassum wightii* (A1, IC_{50}-63.65µg). The algae extracts (S4 and S3) showed antiproliferative activity on A431 cells (Fig-5b).

The ethanol extract of brown seaweed *Turbinaria ornata* (S4, IC_{50}-44.42µg), the water extract of brown seaweed *Turbinaria ornata* (S3, IC_{50}-87.48µg). The methanol extract of *Hydroclathrus tenuis* (H2) and water extract of *Sargassum wightii* (A1) did not show antiproliferative activity on A431 cells. All the 4 algal extracts (S4, S3, H2 and A1) did not show antiproliferative activity on MCF7 cells (Fig-5c). The algae extracts (S4 and S3) showed antiproliferative activity on LnCaP cells (Fig-5d).

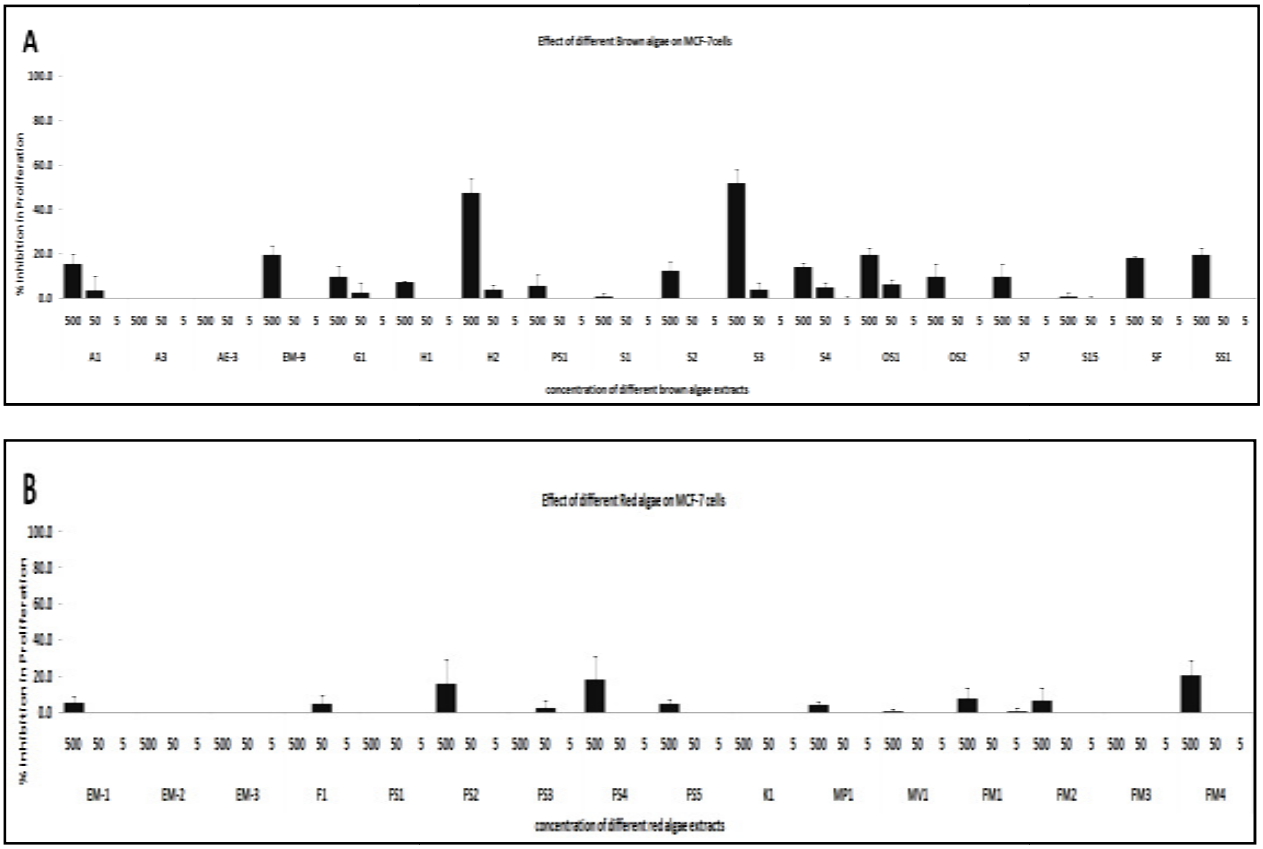

Fig-3: Effect of macroalgae extracts (n=34) A. Brown algae B. Red algae on MCF-7 (Breast cancer) cells

The ethanol extract of brown seaweed *Turbinaria ornata* (S4, IC_{50}-15.73µg), the water extract of brown seaweed *Turbinaria ornata* (S3, IC_{50}-21.36µg). The methanol extract of *Hydroclathrus tenuis* (H2) and water extract of *Sargassum wightii* (A1) did not show antiproliferative activity on LnCaP cells.

Discussion

Identification of anticancer agents that arrest the cancer cell proliferation/growth without inducing toxicity to the normal cells is a major challenge in cancer treatment. It is well established that the marine products from seaweeds have antitumor activity [15]. Antiproliferative screening is a technique to found the anticancer activities of different marine algae. Similar experiments were performed on brown algae *Sargassum fusiforme* and that established as a selective human cancer cytotoxic agent for Ehrlich carcinoma [16]. An antitumor extract from a brown marine alga *Sargassum kjellmanianum* has been reported [17].

In this study, 34 marine macroalgae (brown and red) extracts were studied for their probable anticancer/antiproliferative properties on 4 different types of cancer [NCH-1975 (lung), A431 (epidermoid), MCF-7 (breast) and LnCaP (prostate)] cells. Marine macroalgae extracts used in the present study are from water, methanol and ethanol extracts of brown and red algae and their derivatives. The initial screening was performed in 4 cell types to evaluate anticancer properties of the selected 34 marine macroalgae. Among the 34 macroalgae tested, 4 macroalgae extracts (A1, H2, S3 and S4) showed high antiproliferative activity on NCI-H-1975 (lung) and LnCaP (prostate), moderate anti proliferative effect on A431 (epidermoid) and no antiproliferative effect on MCF-7 (breast) cancer cells.

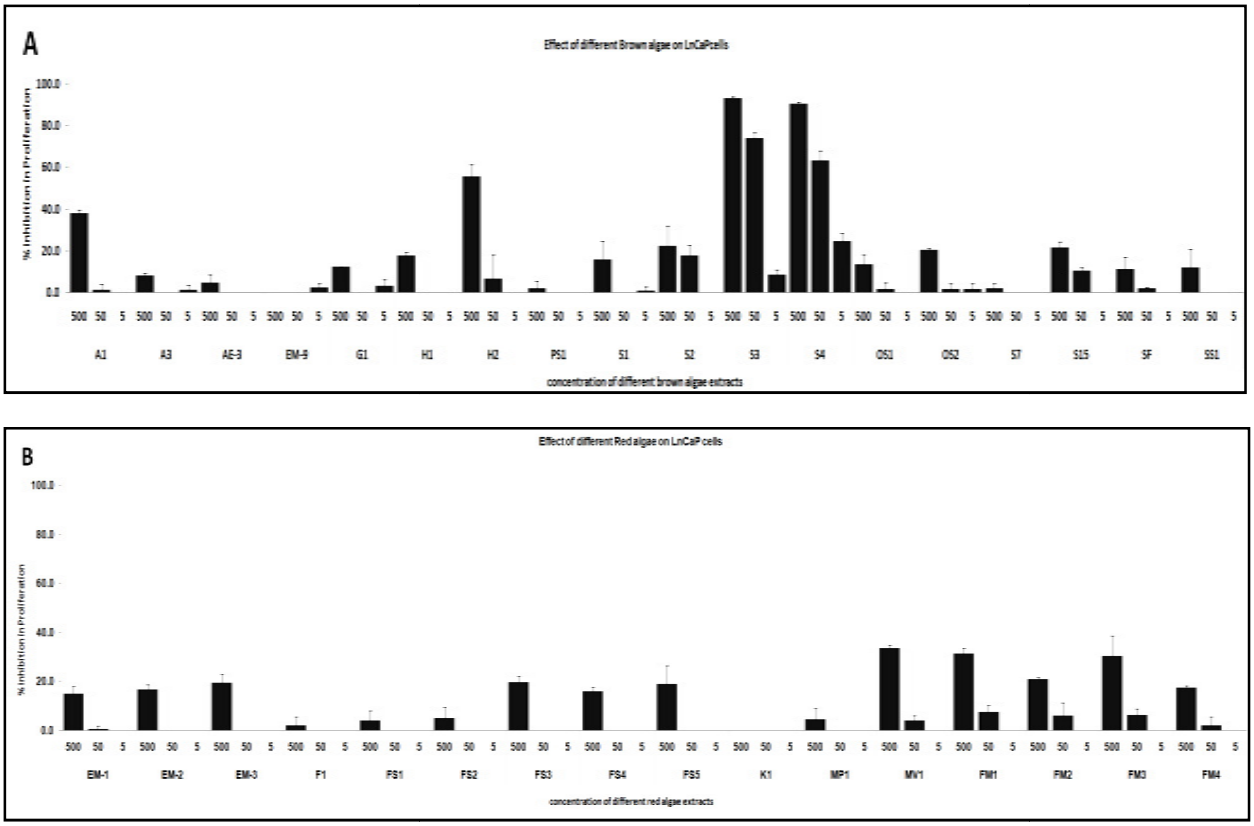

Fig-4: Effect of macroalgae extracts (n=34) A. Brown algae B. Red algae on LnCaP (Prostate cancer) cells

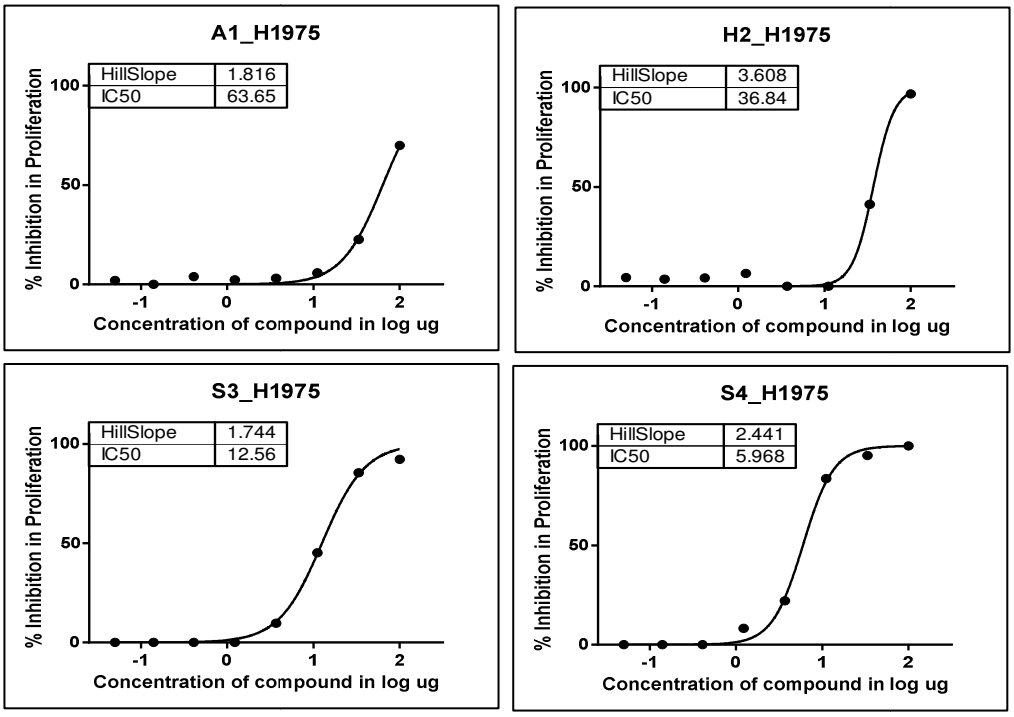

Fig-5a. Determination of IC_{50} value of selected macroalgae extracts on H-1975 (Lung cancer) cells.

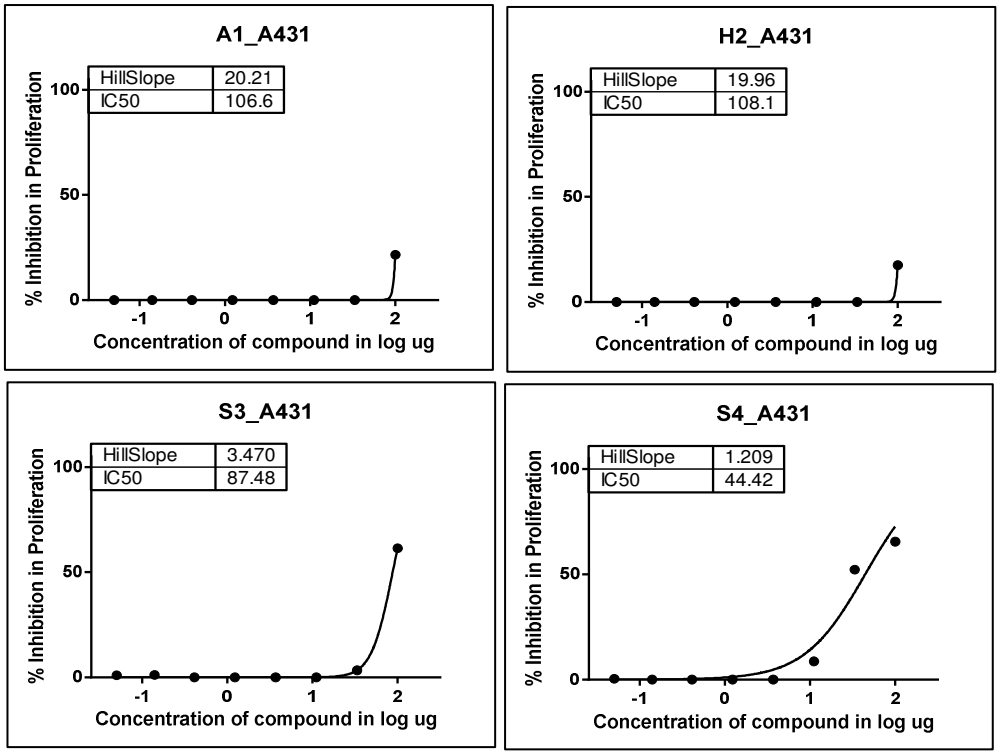

Fig-5b. Determination of IC_{50} value of selected macroalgae extracts on A431 (Epidermoid cancer) cells

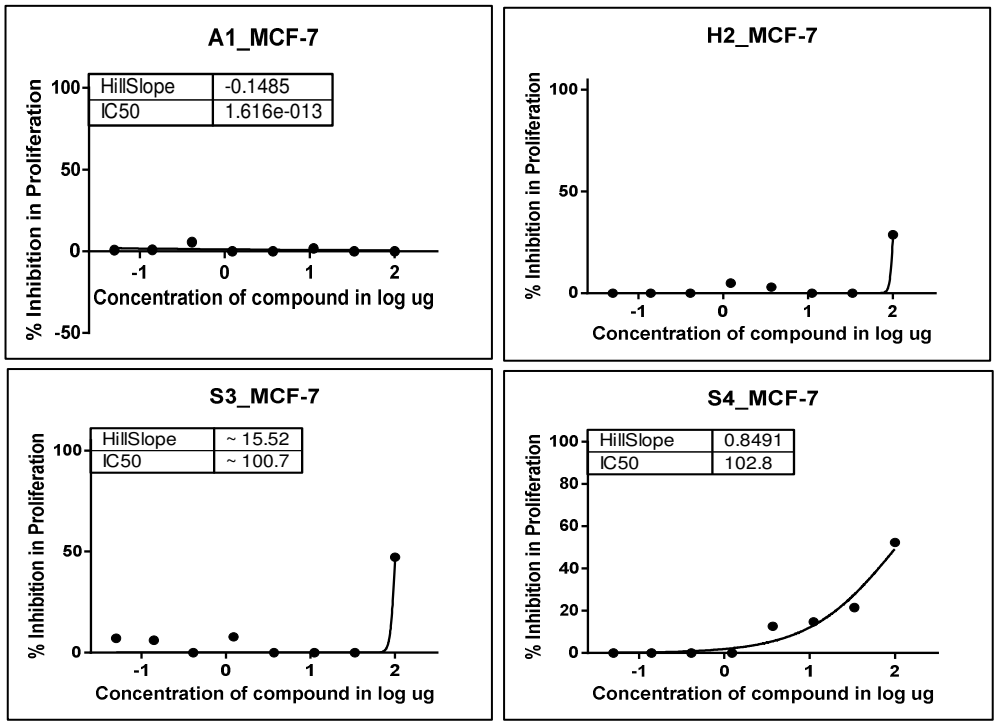

Fig-5c. Determination of IC_{50} value of selected macroalgae extracts on MCF-7 (Breast cancer) cells

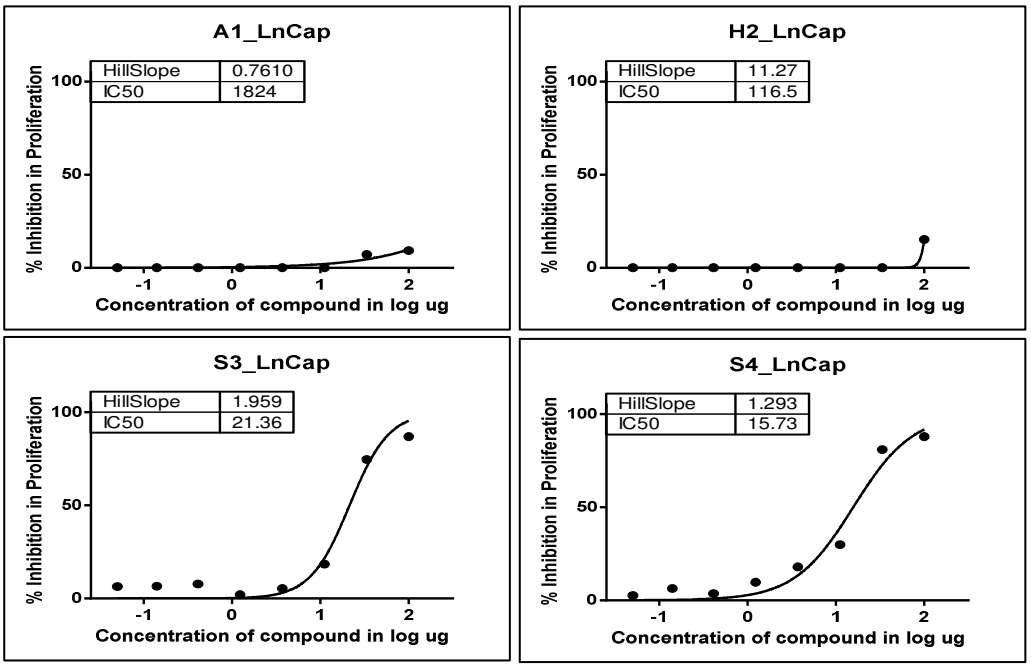

Fig-5d. Determination of IC_{50} value of selected macroalgae extracts on LnCaP (Prostate cancer) cells

Based on the preliminary data we have further determined the IC_{50} of selected marine macroalgae extracts on 4 different cancer cell lines. Our data demonstrated that remarkable antiproliferative activity on lung and prostate cancer cells by the brown seaweed *Turbinaria ornata*. Several studies demonstrated antiproliferative activity of marine macroalgae *Sargassum kjellmanianum, Scytosiphon lomentaria,* and *Sargassum fusiforme* [18]. Our study demonstrated that the brown marine macroalgae *Turbinaria ornata* possessed potent antiproliferative properties against cancer cell types especially against lung and prostate cancers.

The current study established that the ethanol and water extracts of brown seaweed *Turbinaria ornata* have remarkably reduced the growth of the cancer cells. Therefore, this could be the potential candidate for further research to isolate the bioactive compounds and for *in vivo* studies with selective anti cancer activity against human cancers.

Conclusion

In conclusion the selected marine macroalgae extracts showed potent antiproliferative activity against lung and prostate cancers. Therefore they could be utilized as potential therapeutic candidate against several cancers.

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Conflicts Of Interest

The authors declare no conflict of interest.

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