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Original Article

SEAWEED EXTRACTS EXHIBIT ANTICANCER ACTIVITY AGAINST HeLa CELL LINES

ASHWINI S.[1], SURESH BABUT V.[2], SARITHA[3], MANJULA SHANTARAM[4]

Department of Studies in Biochemistry, Mangalore University, Post Graduate Centre, Chikka Aluvara, 571232, Kodagu, Karnataka, India
Email: manjula59@gmail.com

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ABSTRACT

Objective: This study was conducted to examine the anticancer activities in the extracts of marine seaweeds *Gracilariacorticata*.

Methods: The acetone, chloroform, ethanol, methanol and aqueous extracts of collected seaweeds were tested for their anticancer properties *in vitro* against HeLa cancer cell lines.

Results: The anticancer activity of the seaweed extracts was observed at 24hours, 48 hours and 72 h in which chloroform and ethanol extracts of *G. corticata* showed a greater activity with an IC_{50} value of 341.82 µg/ml and 244.7 µg/ml respectively for 48hours. *P*-values were determined by two-way analysis of variance (ANOVA). The morphology of the treated cells showed a great variation when compared to the control cells. Thus, the *in vitro* assay indicates that the extracts of seaweeds are the significant source of a noble anticancer agent.

Conclusion: This study also infers that *G. corticata* could be a potential candidate for cancer therapy in the near future.

Keywords: Seaweeds, Cytotoxicity, HeLa cell lines, Anticancer

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INTRODUCTION

Marine algae (Seaweeds) are a group of marine multicellular algae having various health benefits and biomedical applications in the marine ecosystem. Seaweeds are immensely potential as a supplement in functional food and for the extraction of compounds. They are well known for their richness in minerals, certain vitamins and polysaccharides, but they also contain bioactive substances like proteins, lipids and polyphenols, with antibacterial, anticancer, antioxidant, antifungal, antiviral properties and so on [1, 2].

Seaweeds, which are abundant sources of bioactive components, have gained much importance and interest in recent times. The complex polysaccharides from the brown, red and green seaweeds possess broad spectrum therapeutic properties. The marine environment contains a different number of plants, animals and microorganisms which have a wide range of natural products.

The well documented bioactive metabolites of marine algae include brominated phenols [3], sterols, amino acids and amines [4] and sulphated polysaccharides [5-7]. Recent findings evidenced that seaweeds contained antiviral [8], antibacterial [9], antifungal [10] and anti-tumoral [11] potentials, among numerous others. As an essential goal to lower side effects on immune system, the discovery and identification of new antitumor drug from natural resources have become necessary [12]. Some researchers have described a wide range of biological activities for algal compounds including anti-HIV, anticoagulant, anticonvulsant, anti-inflammatory, bacteriostatic, antineoplastic, and cytotoxic activities [13].

Hundreds of potential anti-tumor agents have been isolated from marine origin especially from marine algae [14, 15]. Isolation of cytotoxic anti-tumor substances from marine organisms has been reported by several authors during the last 40 y [16]. In this study, the *in vitro* antitumor activity of *Gracilariacorticata* a red alga which is found in many parts of the world such as China sea, Indian Ocean, Persian Gulf was determined.

MATERIALS AND METHODS

Collection of seaweed sample

Seaweeds were collected from the rocks of Surathkal beach (13 00`34.1" N lat. and 74 47`16.1" E long.), Dakshina Kannada district, Karnataka. Samples were washed with freshwater to remove adhering debris and identified as *Gracilaria* sp. By Dr. C. R. K Reddy, CSIR-Central Salt and Marine Chemicals Research Institute. The collected samples were transferred to the lab in a polythene bag, shade dried and powdered.

Seaweed extraction

Fifty grams of powdered seaweed was extracted successively using Soxhlet extractor sequentially with different solvents of increasing polarity namely: chloroform, acetone, methanol, ethanol, and water until the extract was clear. The resulting pasty extracts were stored in a refrigerator at 4 °C for future use.

Cancer cell line and chemicals

Cancer cell line HeLa was purchased from National Centre for Cell Science (NCCS), Pune, India. Dulbecco's Modified Eagle's Medium (DMEM), Trypsin-EDTA, Fetal Bovine Serum (FBS), 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide (MTT), sodium bicarbonate, Dimethyl sulphoxide (DMSO) and antibiotic solution were purchased from Himedia. 96 well plates, 6 well plates, Tissue culture flasks (25 and 75 mm^2), centrifuge tubes (15 and 50 ml) were purchased from Himedia.

In vitro assay for cytotoxicity activity (MTT assay)

HeLaHuman cervical cancer cell lines obtained from (NCCS) Pune were maintained in RPMI-1640 supplemented with 10% FBS, penicillin (100 U/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

In vitro assay for cytotoxic activity of investigated extract was performed when the cells reached 70–80% of confluence [17]. A stock solution of the extract was dissolved in the corresponding medium to the required working concentrations.200µl cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent. The cells were allowed to grow for about 12 h. Then, cells were incubated in the presence of various concentrations of the samples (50, 100, 150, 200, 250 µg/ml) for 24hours, 48hours and 72hours at 37 °C in 5% CO_2 atmosphere. The effect on cancer cell survival was determined 24hours, 48hours and72 h after the addition of extract, by the MTT test. Standard drug Berberine was used as a positive control.

Briefly, 20 µL of MTT solution (5 mg/ml of total volume) was added to each well and incubated for a further 3h at 37 °C in 5% CO_2 and humidified the air. Subsequently, 100 µl of solubilization solution (DMSO) was added to solubilize the formazan crystals formed from MTT after the conversion by mitochondrial dehydrogenases of viable cells. Gentle stirring in a gyratory shaker was done to enhance dissolution. Viable cells were determined by the absorbance at 570 nm with reference at 655 nm. Measurements were performed 3 times, and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with an ELISA reader. All experiments were performed in triplicate. The effect of the seaweed extracts on the proliferation of human cervical cancer cells was expressed as the % cell viability, using the following formula:

% Cell viability = A570 of treated cells/A570 of control cells × 100%.

Morphological changes

The plates were observed under an inverted microscope (Biolink) to detect morphological changes. The result showed that HeLa cell proliferation was significantly inhibited by the seaweed extracts. These results indicate that the sensitivity of human cervical cancer cell line for cytotoxic drugs was higher for chloroform and ethanol extracts compared to other extracts.

Data analysis

The IC_{50} values (concentration at which 50% of cells were dead) are reported as mean±standard deviation of three independent experiments. The IC_{50} values against the HeLa cancer cell lines were calculated for all the seaweed extracts inhibiting at least 50% inhibition when tested at concentration. Two-way analysis of variance (ANOVA) was used to compare data using GraphPad Prism version 5.0 software at a 95% confidence limit.

RESULTS AND DISCUSSION

Antitumor activity of seaweed extracts and *in vitro* cytotoxic effect of extracts on HeLa cells

To examine potential cytotoxic effects of seaweed extracts on human cervical cancer cell lines, they were cultured for 24hours, 48 h and72 h at various concentrations of alga extract and analysed by MTT assay (fig. 1, 4). The activity against cancer cell lines is one of the most important specificities of marine algae, and many algae have shown cytotoxic and antitumor activities. In this study, the extracts of *Gracilariacorticata* showed a significant number of cell death of HeLa cells. The percentage of viable cells was calculated using the formula based on which the IC_{50} value of chloroform extract was found to be 341.82 µg/ml (fig. 2a), IC_{50} value of ethanol extract was found to be 244.7 µg/ml (fig. 2b) for 48hours. Morphological changes were determined by inverted microscope (fig. 3a, 3b, 3c and 3d).

It has previously been reported that lophocladines, naphthyridine alkaloids, isolated from the marine red alga *Lophocladia*sps. has exerted inhibitory effects on NCI-H460 lung cancer cells [18]. Several cytotoxic compounds such as fucoidans, laminarians, and terpenoids stated to possess anticancer, antitumor, and antibacterial and antiproliferative properties are reported to be abundant in seaweeds [19]. Hence, in recent years, the search for the cancer therapeutics from natural products has been on the rise. Bioactive compounds in marine plants have been reported against various cancer cell lines.

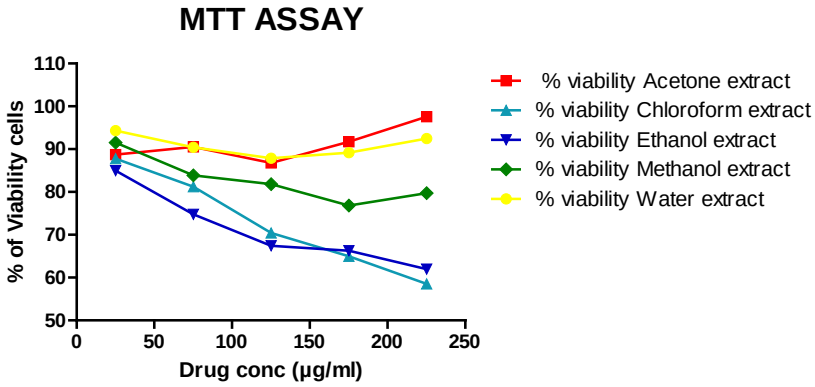

Fig. 1:

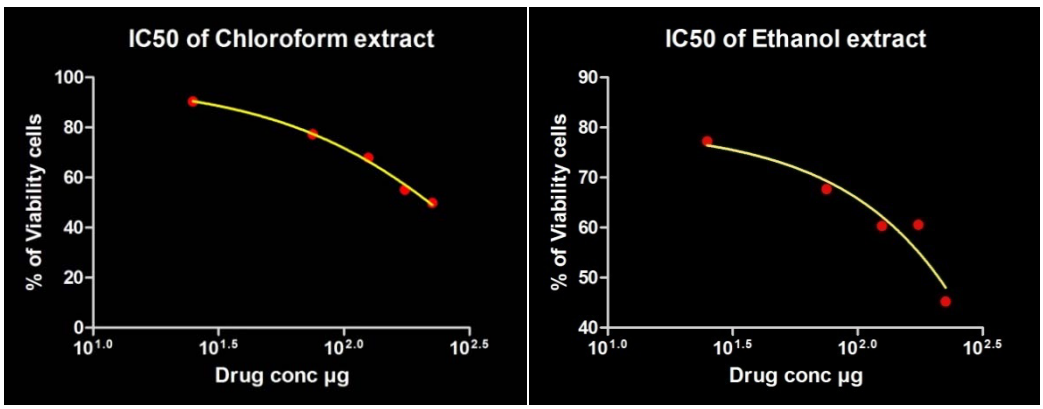

Fig. 2(a-b)

Morphological study

Upon treatment with five different seaweed extracts, a morphological observation of the HeLa cell lines shows the onset of shrinkage.

The cell shrinkage increased progressively with dose and time, and this shrinkage may be due to the growth inhibitory effect of seaweeds.

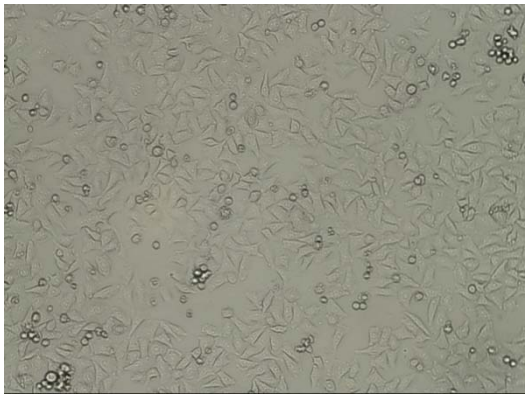

Fig: 3: (a) Untreated HeLa cells

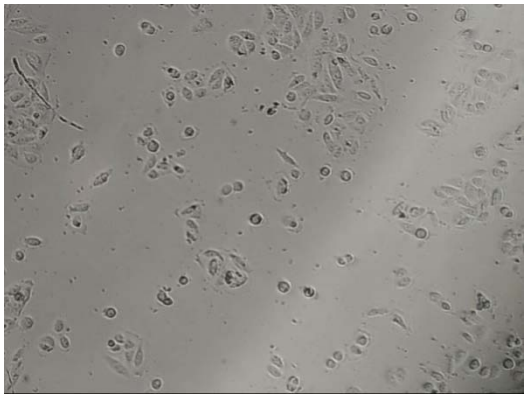

Fig: 3: (b) Chloroform extract treated cells

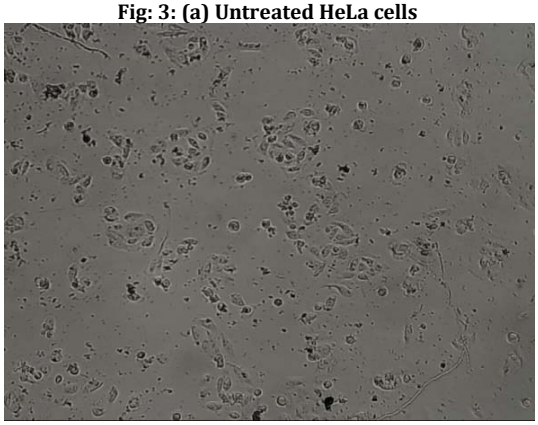

Fig: 3: (c) Ethanol extract treated cells

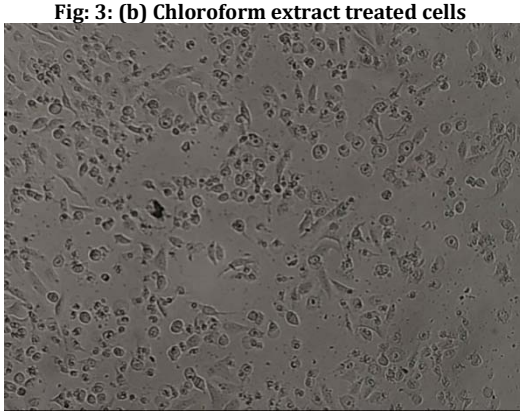

Fig: 3: (d) Berberine extracts treated cells

MTT ASSAY AGAINST HeLa CELL LINES

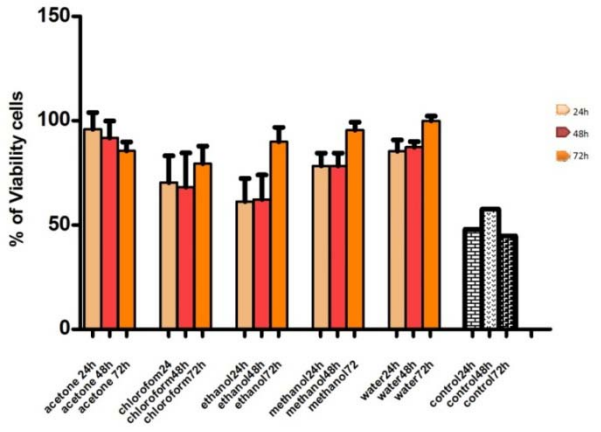

Fig. 4: Bars represent mean cell viability normalised to untreated control cells and error bars indicate SD of three independent experiments

CONCLUSION

Cancer is a group of diseases which is characterised by uncontrolled growth and spread of abnormal cells. The spread of these abnormal cells must be controlled, failing may result in death. Despite considerable progress in research, cancer remains one of the high-ranking causes of death in the world. As an urge to study the effect of the extracts of marine alga *Gracilariacorticata* as a novel therapeutic agent, they were characterised for their cytotoxic effects against HeLa (human cervical cancer) cell lines. To conclude, this extract induces a concentration-dependent inhibition of cells. Based on these results, further studies could be carried out as a search for new compounds from red algae to develop alternative therapeutic measures against diseases.

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CONFLICT OF INTERESTS

Declared none

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