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ORIGINAL ARTICLE

In vitro Antiproliferative activity of Seagrass *Halodule pinifolia* (Miki) on MCF7 Human Breast Cancer Cell Line

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ABSTRACT
Halodule pinifolia is one of the abundant seagrass found along the Vellar estuary. Parangipettai, Tamil nadu, India. In vitro antiproliferative test was performed by MTT (methylthiozoltetrazolium) assay method against MCF7 human breast cancer cells. The ethyl acetate fraction showed that toxicity against cancer cells whereas in normal vero cells no such changes were observed. Ethyl acetate extract of H. pinifolia recorded the maximum antiproliferative activity. Inhibitory concentration (IC50) value of the MCF7 cell line was 66.68 µg/ml. The present findings suggest the possible pharmacological applications of selected seagrass that can be used as food further investigation into the potential used of H. pinifolia as an alternative treatment for cancer.
Keywords: *Halodule pinifolia, Antiproliferative activity, MTT assay, Inhibitory Concentration.*

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INTRODUCTION

New trends in drug discovery from natural sources emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities. These entities are the source of new lead for treatment of many diseases such as cancer, AIDS, inflammatory condition, arthritis, malaria and large variety of viral, bacterial, fungal diseases [1, 2]. The biodiversity in marine environment for exceeds that of the terrestrial environment, research in to the use of marine natural products has pharmaceutical agents is still in its infancy [3]. Given that chemically mediated disease resistance is well documented among terrestrial plants [4], marine plants are known to produce a large number of structurally diverse secondary metabolites [5, 6].

Sea grass is an angiosperm that lives in marine or brackish environment. It represents a unique flora adapted to rigorous salinity, immersion, occasional desiccation, anchorage on the seabed and hydrophilic pollination. Habitats of sea grasses are known to be highly productive and play an important ecological role as nursery grounds for fish and crustaceans such as shrimps, as food source and shelter for many organisms and in recycling of nutrients [7]. In our previous study, Girija *et al*. [8] reported *H. pinifolia* extract had highest antioxidant activity. Therefore the present study was undertaken to evaluate the *invitro* antiproliferative activity of the ethyl acetate extract from seagrass *H. pinifolia*.

MATERIALS AND METHODS

Sample collection and preparation

Halodule pinifolia (Miki) den Hartog were collected from Tuticorin coast, India. The collected seagrass was shade dried and grounded to fine powder. Collected sample was immediately brought to the laboratory in new plastic bags containing natural sea water to prevent evaporation. Plants were washed thoroughly with tap water to remove extraneous materials and shade dried. Dry plant material was ground in an electric mixer and stored at 4ºC until future use.

Extraction of phenolic compounds

Extraction of phenolic compounds followed by Connen *et al*. [9]. Fine powder of dry seaweed (25g) was extracted with 450 ml of methanol at room temperature for 24 h. The extraction procedure was repeated thrice and the extract was filtered through Whatmann No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure using a rotary evaporator (crude extract). This extract was washed with

hexane and dichloromethane (three times each) in order to eliminate lipids and pigments and most of the mannitol, and then extracted with ethyl acetate (three times each). The ethyl acetate phase (crude) was concentrated and stored at 4ºC until future use.

Antiproliferative studies

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% Fetal Bovine Serum (FBS). All cells were maintained at 37^0 C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week. The monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) except the sample 'PC', which is dissolved in phosphate buffered saline (PBS). The samples were then diluted to twice the desired final maximum test concentration with serum free medium. Additional four, 2 fold serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. Following drug addition the plates were incubated for an additional 48 h at 37^0 C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

In vitro cytotoxicity assay (MTT assay)

MTT assay was followed by Mosmann, 1983 [10]. 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.

% cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Data analysis

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

RESULTS AND DISCUSSION

In the present study, ethyl acetate fraction was tested against MCF- 7 breast cancer cells at various concentrations Fig.1. Based on the potentials of the *H. pinifolia* was used against vero normal cell line for their cytotoxicity activity. The active compound showed toxicity against cancer cells whereas in normal vero cells no such changes were observed. The cytotoxicity assay was assessed by the morphological characteristics of the cells such as rounding of cells, shrinkage, aggregation, cell death etc., and it was observed through phase contrast microscope (Fig.2). The cytotoxicity of natural products is based on the presence of antitumor metabolites. Cytotoxic activity of the sample could be related to presence of diterpenes, phlorotannins [11] and sulfated polysaccharides [12, 13].

Several studies has advocated the role of antioxidants in cancer regression, consumption of food rich in antioxidants, reduces many types of cancer [14]. *In vitro* antiproliferative test was performed by MTT assay method against the MCF 7 cells. Cells were cultured for 48 hrs in the presence of different concentrations of compounds and percentage of cell viability was evaluated. The concentration of drug causing lethality to 50% of the cells (LC_{50}) was calculated. The IC50 value of ethyl acetate fraction on MCF-7 cell line was 66.68 µg/ml. No informations noticed in previous literatures on the antiproliferative activity of *H. pinifolia*.

Fig. 1. Cell viability of MCF 7 cells treating with various concentrations of compound)

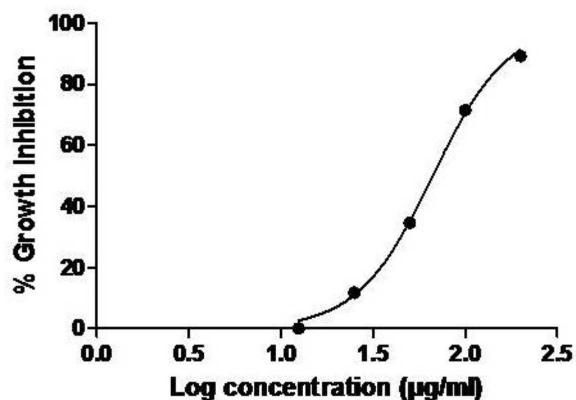
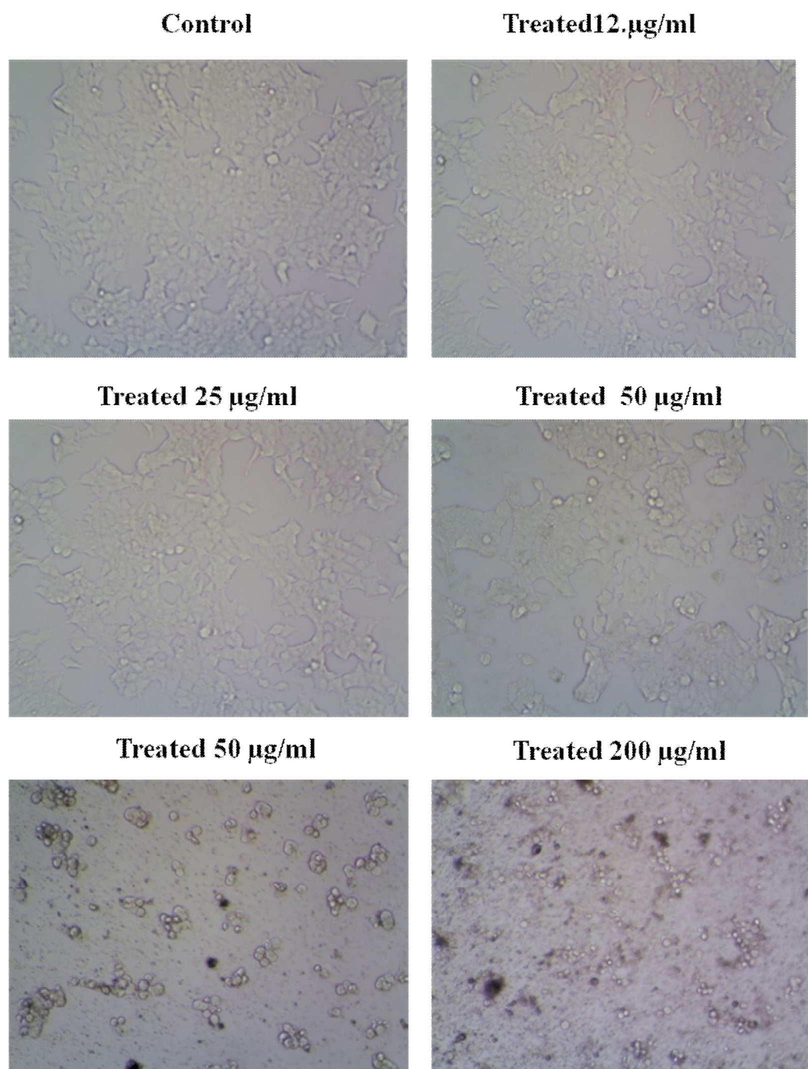


Fig. 2. Morphological changes on Breast cancer cell line (MCF 7) after treating with various concentrations



CONCLUSION

To the best of our knowledge, the present study is the first report on the antiproliferative activity of *H. pinifolia* of velar estuary, parangipettai, Tamilnadu, India. Further bioassays, purification and structural

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characterization of these biological metabolites will yield noteworthy information about their usage in pharmaceuticals, cosmetics and food industry.

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