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In vitro cytotoxic studies of red algae *Portieria hornemannii* and *Spyridia fusiformis* against Dalton's lymphoma ascite and Ehrlich ascite carcinoma cell lines

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ABSTRACT

Objective: To study the *in vitro* cytotoxic activities of methanol extract of *Portieria hornemannii* (*P. hornemannii*) and *Spyridia fusiformis* (*S. fusiformis*) using Dalton's lymphoma ascite and Ehrlich ascite carcinoma cell lines.
Methods: The effect of cytotoxicity of *P. hornemannii* and *S. fusiformis* was evaluated with the concentrations (100 to 200 μg/mL) and assessed for the antitumour activity *vs.* the selected cell lines using Trypan blue assay.
Results: The methanol extracts of *P. hornemannii* and *S. fusiformis* showed potent cytotoxic activity with IC_{50} values of (209.00 ± 0.05) μg/mL and (190.00 ± 0.05) μg/mL against the Dalton's lymphoma ascite cell line and IC_{50} values of (190.00 ± 0.05) μg/mL and (182.00 ± 0.05) μg/mL against the Ehrlich ascite carcinoma cell line respectively. *In vitro* cytotoxicity against the tested cancer cell lines showed strong activity by the abnormal activities of algal residue in the normal cells.
Conclusions: The methanol solvent residue of red algae (*P. hornemannii* and *S. fusiformis*) could be a good candidate. It would be a novel marine resource as a antitumor medicine demonstrated by cytotoxic studies that the above marine algae can be a potential candidate sources as antitumor drugs

1. Introduction

Cancer is the second most dangerous cause of death and its increasing risk coupled with many health problems which is ranked after heart disease in the ultra modern world. At present, fewer potential medicines are available for treatment of various cancer and are causing side effects on some instances. The natural products obtained from marine algae have assumed a vital part in the improvement of a few clinically valuable anticancer managers.

Marine seaweeds are very interesting source of bioactive compounds with diverse biological activities, which have severe effects on the immune system and against cancer incidents.Various studies reported that utilisation of several seaweeds considerably reduced the occurrence of carcinogenesis *in vivo*[1-3]. Alekseyenko *et al*. suggested that polysaccharide of *Fucus evanescens* possesses antitumour and antimetastatic activity in C57BI/6 mice with transplanted Lewis lung adenocarcinoma[4]. *Grateloupia longifolia* prevents angiogenesis in human microvascular endothelial cell line-1, which causes by the presence of polysaccharide isolated from the plant[5]. However, no study of the anti-tumour activity by the chosen algal extracts has been reported so far.

The search for novel substances of natural origin with potential application as drugs for chemotherapy with enhanced cytotoxic activity has gained much attention. The prevention of cancer cell proliferation and induction of antiinflammatory responses by some of the phytochemicals such as flavonoids, polyphenol rich extracts and isolated plorotannins have been reported earlier[6,7].

In addition to other natural products, the seaweeds also have substantial importance paid to the anticancer activity. Marine algae have shown cytotoxic and antitumour nature[8,9]. Seaweed and their organic extracts exhibited cytotoxic activity which also showed in several literatures that highlighted the potential nature of marine products and *in vitro*, as well as *in vivo* anti-proliferative activity in human cancer cell lines[10,11]. Subbiah and Sundaresan reported the antitumour activity and antioxidant activity of methanol residue of *Portieria hornemannii* (Lyngbye) Silva (*P. hornemannii*) and

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Spyridia fusiformis (Wulfen) (*S. fusiformis*) against Dalton's lymphoma ascite (DLA) induced tumour[12]. Similar kinds of observations have been made by Abirami and Kowsalya[13] in Ulvalactuca and Sundaram *et al*.,[14] in Gracilariaedulis.

The breakthrough of novel anticancer drugs which can show cyctotoxic activity only on tumour cells without affecting normal cells will be an paramount task[15]. In the present study, cytotoxic activity of methanol extracts of red algae (*P. hornemannii* and *S. fusiformis*) was tested against DLA and Ehrlich ascite carcinoma (EAC) tumour cells.

2. Materials and methods

2.1. Collection and extraction of seaweeds

Fresh materials of *P. hornemannii* and *S. fusiformis* were collected from intertidal regions of Leepuram, Kanyakumarai, South East Coast of Tamilnadu, India and were identified by standard manual[16]. Fresh materials were washed to remove from epifits, sediment and other organic matter for several times with sea water. The shade dried seaweeds were powdered (yield 7%) and then stirred in a suitable solvent (methanol) (1:20, w/v) for 24 h and the extract was isolated from the methanol portion through filtration. The remaining solid remainder was subjected to repeated extraction using the same solvent. The collected filtrates were concentrated to get the crude solvent extract of algae in the experiment. The crude extract powder was stored at room temperature.

2.2. Effect of methanol extracts of P. hornemannii and S. fusiformis on human cancer cell lines (DLA and EAC)

2.2.1. Propagation of DLA and EAC cell line

In the present investigation, two human carcinoma cell lines, DLA and EAC were acquired from Amala Cancer Research Center, Thrissur, Kerala. They were cultured in an identical cell suspension in the peritoneal cavity of the mice. By using serial transplantation method from mice to mice, the cells were maintained. From the donor mice carrying tumor for 7–9 days, the DLA and EAC ascitic fluid were drawn out for further studies. The phosphate buffer saline (pH 7.4) was used to thoroughly wash the fresh cells and was repeated thrice which drawn from the intraperitoneal cavity and diluted in phosphate buffer saline to gain a concentration of 1×10^6 cells/mL and used for the *in vitro* cytotoxic studies.

2.2.2. Trypan blue exclusion test

The viability of cells before and after treatmenting with experimental algal residue was quantized by using trypan blue exclusion test, as a semi-quantitative method. Trypan blue was termed as vital dye and it stained the dead and damaged cell. The nuclei of the damaged cells readily taken up the dye and appeared as blue in colour. Whereas, the viable cells with intact cell membrane were impermeable to trypan blue dye. The malignancy cells were planted at a concentration approxiamately 2×10^4 cells per well with various concentrations of extract at 37 ℃ in the 5% CO_2 atmosphere. Equal volume of medium (20 µL) and trypan blue (20 µL) were mixed and viable and dead cells were counted by Neubauer haemacytometer after 72 h incubation[17].

Both the malignant cells (1×10^6 cells/mL) added at various concentrations starting from 10 to 200 µg/mL of the methanol residue of *P. hornemannii* and *S. fusiformis* were made up to 1 mL as a final volume for 48 h and incubated at 37 ℃. After the incubation, 100 µL of 0.4% trypan blue dye was added to each of the Eppendrof containing DLA and EAC cell line and the cells were allowed to take up the stain for 2–5 min. The stained cell suspensions were loaded in the counting chamber of the haemocytometer and the stained and unstained cells were calculated using the following formula and results were represented as percentage of dead cells.

Dead cells (%) = (No. of dead cells/Total No. of cells) × 100

3. Results

3.1. In vitro cytotoxic activity

3.1.1. Evaluation of cytotoxicity and determination of IC_{50} values

Trypan blue dye exclusion assay method was used to evaluate *in vitro* cytotoxicity assay of the methanol extracts of the marine red algae (*P. hornemannii* and *S. fusiformis*). The two different cell lines, DLA and EAC were treated with the methanol extracts of *P. hornemannii* and *S. fusiformis* with various concentrations ranging from 10 to 200 µg/mL. The cytotoxicity study of the seaweed extracts against DLA and EAC malignancy cells were presented in Figures 1 and 2. From the assay study, the most effective concentration with IC_{50} values against proliferation of cancer cells was calculated from the number of dead cells after treating by algal extracts.

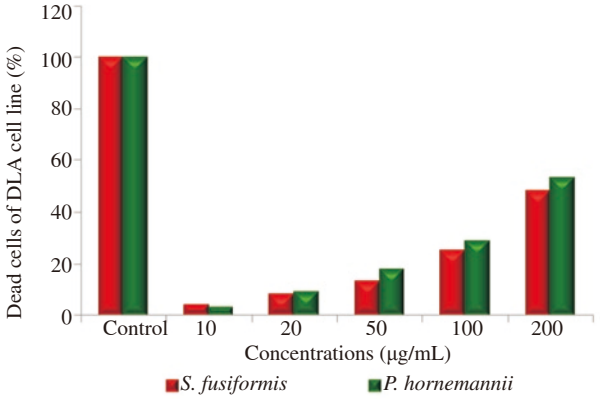

Figure 1. Effect of methanol extract of experimental algae against DLA cell line.

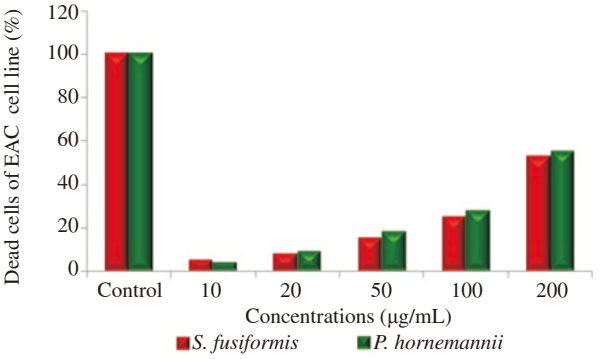

Figure 2. Cytotoxic activity of methanol extract of experimental algae against EAC cell line.

The cytotoxic activity of these algal extracts was found to be dose-dependent and the effects were observed microscopically at the end of the study. In the current investigation, it was noticed that, with an increase in concentration of seaweed extract from 10, 20, 50,

100 to 200 μg/mL caused a gradual decrease in the cell viability which was clearly observed by calculating the number of dead cells after treating with algal extracts. The most effective concentration (200 μg/mL) of *P. hornemannii* exhibited 53% and 55% of cell death against DLA and EAC respectively. Whereas, in the case of *S. fusiformis*, 48% of cell death was observed in DLA and 53% in EAC (Figures 1 and 2). From these results, it was observed that the crude methanol extracts of *P. hornemannii* and *S. fusiformis* exhibited exceptional cytotoxic activity with the IC_{50} values of (209.00 ± 0.05) μg/mL and (190.00 ± 0.05) μg/mL against the DLA cell line and IC_{50} values of (190.00 ± 0.05) μg/mL and (182.00 ± 0.05) μg/mL against the EAC cell line (Figures 3 and 4). Based on the observation, the present study suggested that DLA and EAC cells of the negative control wells (without extract) were increased after 72 h.

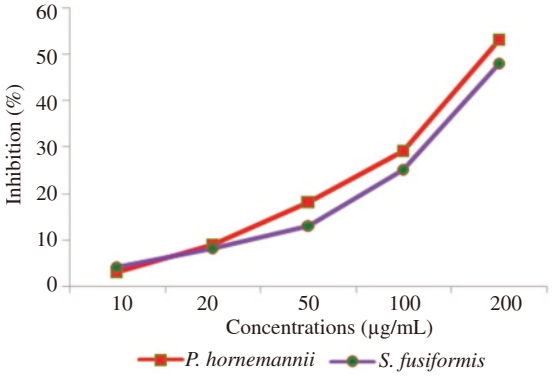

Figure 3. IC_{50} values of methanol extract of *P. hornemannii* and *S. fusiformis* against DLA cell line.

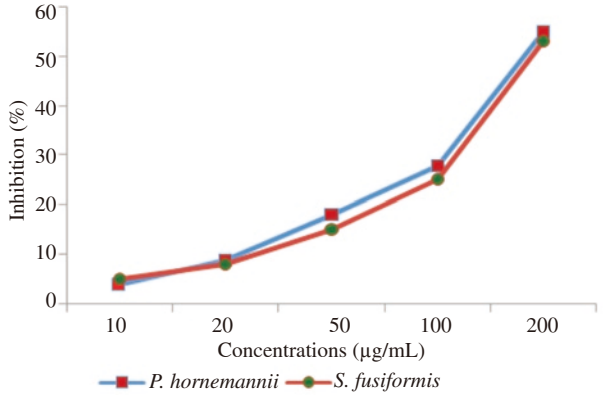

Figure 4. IC_{50} values of methanol extract of *P. hornemannii* and *S. fusiformis* against EAC cell line.

4. Discussion

The seaweeds obtained from tropical and sub-tropical environments accountted for more than 2400 natural products isolated from them[18-22]. The traditional chinese medicines derived from algal sources have also been tremendously contributed to the treatment of cancer[23]. The structure of bioactive substances obtained from algal materials have been elucidated using modern analytical methods and reported by many research groups[24,25]. Many marine algal sources showed exceptional anti-cancer activity against wide spectrum of cancer cells as reported[8,26].

A rapidly growing carcinoma cell line is Ehrlich ascites tumour gained importance because of its very aggressive behavior[27]. The EAC cell lines hosed intense formation of edema with increased ascitic fluid formation which is essential nutrition for tumor cell growth[28,29]. The present study prove that *P. hornemannii* and *S. fusiformis* methanolic residue can lower the ascites tumour in a dosage dependent mode.

The progressive increasing in the concentration of *P. hornemannii* and *S. fusiformis* extract from 10 to 200 μg/mL showed decreased cell density as observed from the intensity of DLA and EAC in the dye assay. Thus, the study revealed that dose-dependent cytotoxic activity of *P. hornemannii* and *S. fusiformis* residue *vs*. cancer cells indicates the effectiveness of the algae. The algal extract treated cancer cells showed cell shrinkage with the formation of apoptotic bodies along with the babbling of cell membrane which supported the cyctotoxic activity of the experimental algae.

Among the bioactive compounds, polysaccharide presented in brown algae, which displayed various biological effects like removal of heavy metals from the body and possessed antitumor and anti-inflammatory property[30]. Many researchers have revealed that the natural products derived from polyphenolic compounds, polyunsaturated fatty acids, terpens, sterols and fucoidans have showed anticancer activity[31-34]. Antitumour and cytotoxic properties of these species belong to four structural types (polyketides, terpenes, nitrogen containing compounds and polysaccharides)[35].

The current investigation offered an evidence that *P. hornemannii* and *S. fusiformis* have *in vitro* anti-cancer efficacy against DLA and EAC cell lines. The potent medicinal properties of these algae may be used for development of effective therapeutic approaches towards the prevention or treatments of various immune conditions and different types of cancer. Thus, the mechanism of selective cytotoxicity is needed for further studies. In order to prove the mechanism and efficacy of *P. hornemannii* and *S. fusiformis* as a potent anticancer therapeutic agent, further investigation on its bioactive components is necessary.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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