Research Article

Formulation, Cultivation and Antiproliferative Activity of Blue Green Algae *Spirulina platensis* - Short Term *In Vitro* Model

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Received: 13-04-2019; **Revised:** 24-05-2019; **Accepted:** 03-06-2019.

ABSTRACT

A disease caused by an uncontrollable division of abnormal cells in a part of the body. Cancer is a group of more than 100 different diseases. It can be developed almost anywhere in the body. First the changes in the genes cause cancer to grow more quickly and live longer. Normally body cell grows and divides into new daughter cells when they need. But cancer cells produce more and more cells when there is no need for new cells and forms cell mass called tumor. *S. platensis* had moderate anti-proliferative against both EAC & DLA cell model. These data support a chemoprotective role of this edible alga with promising potential for its broad use in the chemo treatment of Cancer but adjuvant with dose dependent standard drugs. Further investigation needed to reveal the nutritive constituent that may have reducing and preventing different forms of cancer. It can be concluded from this study that microalgae have the potential to be further developed as an anticancer agent taking into account that it is a product of nature. However further studies need to be performed to fully exploit its anticancer properties such as determination of the nature of cell death and confirmation of apoptosis.

Keywords: Blue Green Algae, Anti-proliferative, In-Vitro Model, *Spirulina platensis*.

INTRODUCTION

A disease caused by an uncontrollable division of abnormal cells in a part of the body. Cancer is a group of more than 100 different diseases. It can be developed almost anywhere in the body[1]. Today, there is much interest in studying natural products and their derivatives in search of options for disease treatment and other medical applications. Natural products are especially notorious as anticancer and anti-infective agents[2]. With the current decline in the number of new molecular entities from the Pharmaceutical industry, novel anticancer agents are being sought from traditional medicines[3]. In last few decades allopathic system of medicine, which was rapidly accepted worldwide, but latter due to its lot of adverse effect, again people step down to alternative medicine for better therapeutic result and safety profile and now the people are more believing in such drug[3]. *Spirulina platensis* have been used for the treatment and also in management of many diseases. Some other algae like red algae (*Gigartina papilatta*) also use as super food by Chinese and Japanese people[4]. But spirulina has more and more benefits over them. It helps in strengthening the immune system, making the body produce more red and white blood cells, as well as the cells, which kill viruses and germs, increasing antioxidant protection, since the super food helps fight free radicals responsible for body aging process[5]. Spirulina promotes body natural cleansing and detoxification[6]. On the basis of literature review and previous work done with this micro algae the objectives set forth are, Formulation of a suitable synthetic medium for high yield of *Spirulina platensis*[7], Cultivation and Extraction of *Spirulina platensis*[8], Estimation of its protein content and short term anticancer activity[9]. Industries for cyano-bacterial or micro-algal products have recently developed several latest technical system for the biomass production and down streamed this biomass into differentiated products[9]. But still research going on for highly nutritive commercial medium for bulk production of *Spirulina platensis*[10].Very importantly keeping in mind the production quantity and economic worth. Also *Spirulina platensis* found to have high therapeutic activity[11].

MATERIALS AND METHODS

Collection of Algae

All the chemicals were procured from Loba chemie Pvt Ltd, Mumbai. *Spirulina platensis* sample was collected from tank of Vivekanandha College of Engineering for Women, Elayampalayam and cultivated in standard Zarrouk medium and formulated synthetic medium. They are represented in figure 1.

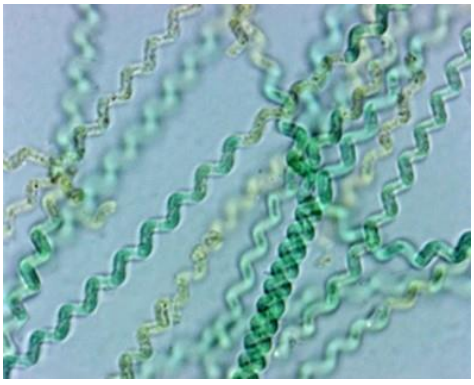

Figure 1: Image of *Spirulina platensis*

Preparation of *Spirulina* Powder

Cultivated *Spirulina* was filtered using voil 100 cloth and the wet mass was collected. Collected *Spirulina* wet mass was dried in room temperature and *spirulina* powder was obtained.

Protein Estimation test by Biuret Method[12]

Take 1 ml of test solutions in dry test tubes and in another tube take 1 ml distilled water as control. Add 1 ml of biuret reagent to all test tubes, mix well. Look for the development of blue colours**.** Measure the absorbance at 540 nm.

Short term *in vitro* cytotoxicity studies in EAC and DLA cell line by Tryphan blue dye exclusion method[13&14]

DLA (Daltons lymphoma ascites) bearing mice, Phosphated buffered saline (PBS), NaCl(4g), Na_2HPO_4(0.72 g), KH_2PO_4(0.1g), KCl(0.1g), Distilled water(500ml), Trypan blue 1% in saline, Heamocytometer. EAC and DLA cells were from Amala Cancer Institute, Thrissur, Kerala, India and were propogated and maintained in intraperitoneal cavity of Swiss albino mice at our animal house.

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out cytotoxicity studies.

Determination of cell viability by tryphan blue dye exclusion method

Cells were aspirated from the peritoneal cavity of tumour bearing mice. The cells were washed 3 times using PBS. The viability of the cell were checked using tryphan blue cell viability should be above 98%. Different dilution of 10^{-1}, 10^{-2} and 10^{-3} were made. The number of cells in the 10^{-3} dilution was counted using heamocytometer and the cell number was adjusted to 1 X 10^{-7} cell/ml. The experiment was setup by incubating different concentration of the drug with 1 X 10^6 cells .The final volume of the same mixture was made upto 1ml using PBS and was incubated at 3°C for about 3hrs.100 microlitre of tryphan blue was added after incubation and number od dead cells was counted using heamocytometer.

RESULTS

Yield

Yield of *Spirulina platensis* in Zarrouk medium was found to be 1.71 gm and in our formulated synthetic medium was 2.52 gm in 20 lit can after 2 successive extraction. Protein Estimation test is found to be the concentration of protein in Sample 1 is 16.5 mg/ml. Protein Estimation test is found to be the concentration of protein in Sample 2 is 21.780 mg/ml. They are represented in Table 1.

Table 1: Protein Estimation Test

S. NO	Solution (0.1 mg/ml)	Absorbance at540nm
1	Blank	0.000
2	Standard	0.2539
3	Sample 1	0.0420
4	Sample 2	0.0553

Short Term *In Vitro* Cytotoxic Activity *Spirulina Platensis* on DLA & EAC Cell Line

Short Term *In Vitro* Cytotoxic Activity *Spirulina Platensis* on DLA & EAC Cell Line is represents in Table 2.

Table 2: Short term *in vitro* cytotoxic activity *Spirulina platensis* on DLA & EAC cell line

Drug Concentration in µg/ ml	Percent cell death (%)	
	DLA	EAC
200	38	46
100	20	24
50	12	18
20	6	10
10	2	6

DISCUSSION

Cyanobacteria of the genus *Spirulina Sp* have been studied not only for because of the potential as a protein source but also because of their therapeutic properties[15].Phenolic compounds have been extensively studied for the antioxidant properties not only in fruits and vegetables but also in Cyanobacteria[16]. For example, *Spirulina platensis* has been reported to have free radicals scavenging properties and thus antioxidant activity[17]. Deng and Chow (2010) demonstrated the hypolipidemic, antioxidant and anti-inflammatory activity of Spirulina. *Spirulina platensis* has been used by humans for centuries as evidenced by reports from Mexico and Central Africa[18]. Currently, it is widely used as a nutraceutical namely for the prevention of diabetes, although numerous other biological effects have been ascribed to this algae. In our study attempt was done for a suitable formulation of medium that may increase the yield value so that it can be considered for small scale production and further for its economical commercial production[19]. First it was cultivated in a standard Zarrouk's medium and it showed a good yield based on pH, temperature and light. Attempt was done for tailoring a medium that was with good nutritive value, so that increased yield would be achieved. Our finding suggest that yield value was improved in our formulated synthetic medium, proved it has good source of electron, carbon and energy for increased growth rate[20]. Based on the literature review our research proceeded towards proving the Antiproliferative activity of *Spirulina platensis*

(Formulated medium) and the work was designed for short term *in vitro* anticancer activity using EAC & DLA model[21]. Tryphan blue exclusion method was selected to determine its antiproliferative activity. Surprisingly we found that *Spirulina platensis* exerted moderate antiproliferative action both on EAC & DLA cell line model[22]. Thus, finding further made as to search for prove and we found that Waleed Barakat and his team has proved *Spirulina platensis* lacks antitumor effect against EAC in female mice by *in vivo* study. Further their findings revealed that when it is combined 5-Fluorouracil it exerted potential Antiproliferative activity which was to be only dose dependent. Protein estimation had no questions for its high protein profile, when used Biuret method. Collectively our data suggest that *Spirulina platensis* showed moderate antiproliferative activity against both EAC & DLA cell line[23].

CONCLUSION

In conclusion, *S.platensis* had moderate anti-proliferative against both EAC & DLA cell model. These data support a chemoprotective role of this edible alga with promising potential for its broad use in the chemo treatment of Cancer but adjuvant with dose dependent standard drugs. Further investigation needed to reveal the nutritive constituent that may have reducing and preventing different forms of cancer. It can be concluded from this study that microalgae have the potential to be further developed as an anticancer agent taking into account that it is a product of nature. However further studies need to be performed to fully exploit its anticancer properties such as determination of the nature of cell death and confirmation of apoptosis.

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Source(s) of support: Amala Cancer Institute, Thrissur, Kerala, India, **Conflict of Interest:** None.