



## Cytotoxicity of Spirulina Extract on Oral Cancer Cell Lines

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### ABSTRACT

The aim of the study is to analyse the cytotoxicity of Spirulina on oral cancer cell lines. Spirulina refers to various species of blue-green algae found naturally in lakes and grown commercially. It is used as a food colouring and is taken orally as a nutritional supplement in tablet, capsule or dried powder form. It is claimed to have immune-stimulating effects and to be beneficial in the treatment of cancer. Spirulina display antitumor activity against many cancers in human and animal systems. Anticancer and antioxidant property of certain medicinal herbs can be used to treat trauma over a longer period of time which is always very promising. Phytochemicals present in this herb may have antioxidant property. Therefore, Spirulina may be used in the treatment of cancer. Further research is required to know the exact mechanism of action of Spirulina.

**Keywords:** Spirulina, Oral Cancer, Cytotoxicity, Anticancer, Antioxidant.

### INTRODUCTION

Medicinal plants have been identified and used throughout human history. In India, the Ayurveda medicinal system is based on herbs. Plants have the ability to synthesise a wide variety of chemical compounds that are used to perform important biological functions, and to defend the body.<sup>1</sup>

Plants involve bioactive secondary metabolites and because of their complex structure, researches in this domain are notably being remarked by scientists.<sup>2,3</sup> Scientists collect different parts of many plants, prepare extracts, and test the extract for finding new and novel chemotherapeutics to treat cancer, as well as viral and microbial infection. Cytotoxic screening of plants is the preliminary methods to identify active compounds of plants.<sup>4,5</sup>

Spirulina refers to various species of blue-green algae found naturally in lakes and grown commercially. It is used as a food colouring and is taken orally as a nutritional supplement in tablet, capsule or dried powder form. It is claimed to have immune-stimulating effects and to be beneficial in the treatment of cancer. Spirulina display antitumor activity against many cancers in human and animal systems.

One of the diseases that dominate today's society is cancer, which is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body.<sup>6</sup> There are over 100 different known cancers that affect humans.<sup>7</sup> Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy.<sup>6,8</sup>

Anticancer and antioxidant property of certain medicinal herbs can be used to treat trauma over a longer period of

time which is always very promising. Phytochemicals present in this herb have anticancer property.

The "phyto-" of the word phytochemicals is derived from the Greek word 'Phyto', which means plant. Therefore, phytochemicals are plant chemicals. Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases.<sup>9</sup> Phytochemicals have the potential to stimulate the immune system, block substances we eat, drink and breathe from becoming carcinogens, reduce the kind of inflammation that makes cancer growth more likely, prevent DNA damage and help with DNA repair, reduce the kind of oxidative damage to cells that can spark cancer, slow the growth rate of cancer cells, trigger damaged cells to commit suicide before they can reproduce and help to regulate hormones thereby resisting the risk of cancer.<sup>1</sup> Thousands of phytochemicals have been identified so far, and scientists have only begun to investigate their promise. Natural dietary phytochemicals have been widely used in vitro, in vivo, and preclinical cancer prevention and treatment studies. Some of these clinical trials have shown various degrees of success.<sup>10</sup>

Cytotoxicity is the degree to which an agent has specific destructive action on certain cells. It is the possession of destructive action, particularly in reference to lyses of cells by immune phenomena.<sup>11</sup>

Cell proliferation rates or viability levels are good indicators of cell health. Proliferation or viability analysis which is crucial for cell growth and differentiation studies, and are often coupled with metabolism analysis.



## MATERIALS AND METHODS

### Preparation of extract

Spirulina was commercially bought. The dried powder (50 grams) was extracted with 100ml of chloroform. The extraction was followed by the filtration process.

### Maintenance of cell lines

The vial containing the KB cell lines procured from ATCC (CCL-17) was removed from liquid nitrogen freezer and immediately placed in a 37°C water bath. It was agitated continuously until the medium thawed. Then it was centrifuged at 1000rpm for 5 minutes at room temperature. The supernatant was discarded and cells were washed with fresh medium to remove residual DMSO. The cell pellet was re-suspended in 3ml of DMEM with 10% FBS. It was then incubated in a CO<sub>2</sub> incubator at a humidified 37°C. The medium was changed every 2-3 days or when pH indicator (e.g. Phenol red) in medium changed colour. The culture was kept in a medium with 10% FBS until cell line were re-established.

### MTT Assay

The MTT assay (Mossmann, 1983)<sup>12</sup> is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO<sub>2</sub>. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X 10<sup>4</sup> cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the samples (100, 200 & 300 µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Cyclophosphamide was used as a positive control.

Cell survival was calculated by the following formula:

$$\text{Viability \%} = (\text{Test OD} / \text{Control OD}) \times 100$$

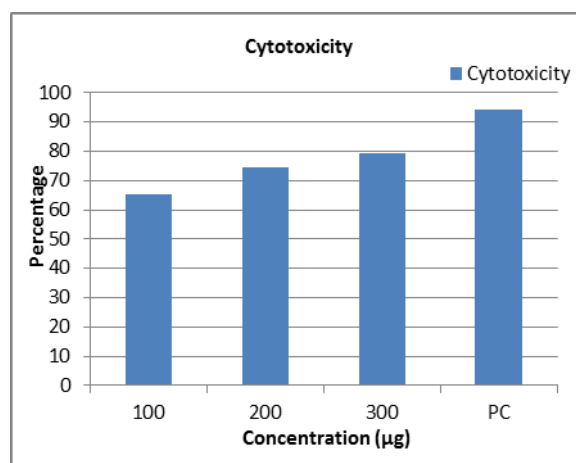
$$\text{Cytotoxicity \%} = 100 - \text{Viability\%}$$

## RESULTS AND DISCUSSION

Cytotoxicity analysis using various concentrations of Spirulina extract (100, 200, 300 micrograms) was performed. The viability of the KB cell lines shows a gradual decrease as the concentration of the extract is increased. This exhibits the Cytotoxicity of Spirulina extract with increasing concentration.

Graph (Figure 1) shows percentage cytotoxicity of Sample and PC (PC – Positive Control)

X-axis: Concentration; Y-axis: Percentage



## CONCLUSION

The use of natural products for treatment is generally a more preferred option due to the adverse side effects which accompany other treatment plans.<sup>1</sup> The presence of these phytochemicals reveals the anticancer property of the extract. The study exposed the cytotoxic potential and antitumor properties of Spirulina, medicinal blue-green algae. The extract presented cytotoxic effect in high concentrations; leading to increased cell death in the KB cell culture.<sup>1</sup> Further research is required to know the exact mechanism of action of Spirulina as an anticancer drug.

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