SCREENING OF ANTICANCER ACTIVITIES OF CRUDE EXTRACTS OF UNICELLULAR GREEN ALGAE (*Chlorella vulgaris*) AND FILAMENTOUS BLUE GREEN ALGAE (*Spirulia plantis) ON SELECTED CANCER CELL LINES*

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Abstract

Biomass of Chlorella vulgaris and Spirulina platensis were extracted using either ethanol or chloroform and screened for their anticancer activities against selected cancer cell lines. The microalgae crude extracts were tested for their effectiveness as anticancer agent on breast cancer cell lines MCF-7 and liver cancer cell lines HepG2. The results were compared with the cytotoxicity effects of microalgae on normal hepatic fetal human epithelial (WRL-68) cell lines. The tests were carried out by means of MTT assay (a colorimetric assay) to determine cell viability by assessing the values of IC_{50}. The result shown that the MCF7 is the cancer cell lines that reach 50% inhibition of cell by both microalgae compared to HepG2. The extraction by chloroform of Chlorella vulgaris indicates that there was 50% inhibition (IC_{50}) of cells on MCF7 much higher compare to the ethanol extraction of both spirulina and chlorella with approximately at 89 µg/ml. the ethanol extraction of Spirulina platensis indicates that there was 50% inhibition (IC_{50}) at 85 µg /ml. Chloroform extract of the unicellular microalgae and ethanol extract of filamentous microalgae exhibited a promising result as anticancer agent as seen by the IC_{50} value obtained. The extract did not demonstrate IC_{50} value on normal cells and can increase cell proliferation. This preliminary study suggests that new anticancer natural products from unicellular green algae and filamentous microalgae are possible.

Keywords: Anticancer agent, Cytotoxic, Unicellular, Filamentous and Cell Proliferation

1. Introduction.

Spirulina, a filamentous cyanobacterium, possesses diverse nutritional and health benefits due to high concentrations of nutrients. Studies show Spirulina preparations increase phagocytic activity of macrophages and stimulate antibodies and cytokines production. It may also faciliate lipid and carbohydrate metabolism. Studies also demonstrate its benefits against several types of virus (e.g. HIV), toxicity and cancers (Khan Z *et al*, 2005 and Blinkova LP *et al.,* 2001). Previous studies found an antitumor substance in the culture supernatant of *Chlorella vulgaris* (CV), a galactose-rich glycoprotein, consisting of a 6-linked β1-6 galactopyranose-rich containing carbohydrate (70%) and protein (35%) (Hasegawa *et al*., 2002). The induction of apoptosis is known to be an efficient strategy for cancer therapy (Hu H *et al*., 2002). Many Chinese herbal remedies such as Rubus coreanum (Kim EJ *et al*., 2005)

and Paeoniae Radix, (Lee SMY *et a*l., 2002) have been demonstrated to possess the ability in triggering the apoptotic pathway (Huang ST.,*et al*. 2003 and Joshi SS., 2000) in HepG2 cells.

Unicellular and filamentous microalgae are photosynthetic organisms with relatively simple requirements for growth, when compared to other sources of biomass. The carbon source necessary for the cultivation of these microalgae represents up to 60% of the costs with nutrients. The microalgae also are one of the richest sources of biomedically relevant compounds with extensive therapeutic pharmaceutical applications (Gademann & Portman, 2008). Microalgae metabolites show an interesting and exciting range of biological activities ranging from antimicrobial, immunosuppressant, anticancer and antiviral to proteinase-inhibiting activities which are striking targets of biomedical research (Luesch *et al*., 2002).

Hence new anticancer agents should be investigated from various resources. A great number of antitumor compounds are natural products or their derivatives, mainly produced by blue-green algae (Patterson *et al*, 1991, 1993). A study will be focus with an alternative treatment to produce new anticancer-type natural products from microalgae. This research was accomplished to explore the ability of *Chlorella vulgaris* (CV) and *Spirulina platensis* (SP) as a potential anticancer agent on different types of cancer cells.

2. Methodology

2.1 Preparation of extracts

Biomass of CV and SP were grinded and dried in oven at 30°C. 50 g of the powdered from samples were then soaked in ethanol and chloroform separately in the ratio of 1:20 for 72 hours. The solution was then subjected to evaporation using the rotary evaporator at 40-48°C. Stock extract solution was prepared by dilution with DMSO to a concentration of 10 mg/mL.

2.2 Cell lines and culture medium

The cell lines (MCF-7, HepG2 and WRL-68) involved was obtained from PROMISE laboratory, Universiti Teknologi MARA (UiTM). The cell were maintained in MEM tissue culture media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cultured cells were incubated in the CO_2 incubator with 5% CO2 supply at 37°C.

2.3. In vitro cytotoxic assay

Confluent cell cultures were harvested with trypsin-EDTA solution and plated onto 96-well plate at cell density of approximately 1 X 10^4 cells/well. Serial dilution of sample was carried out on the plate with the highest concentration of microalgae extract being 100 µg/mL. Each test well was added with 100 µL of the diluted microalgae extract. Later 100 µL of cells to be tested were added to the wells making up the volume to a total of 200 µL of solution. The plates were then incubated at 37C in the CO_2 incubator. The assay was carried out with different exposure times which were 24, 48 and 72 hours. At the end of the incubation period, 20 µL of MTT solution were added to each test well. The plate was later incubated for 3 to 4 hours to allow the reaction to take place. Following incubation, most of the solution in each well was discarde leaving the purple formazan precipitate at the bottom of the well. Then, 100 µL of DMSO was added to each well and the solution was pipetted thoroughly to dissolve the purple formazan crystals. The amount of formazan produced after treatment was read using a microplate ELISA reader at the wavelength of 570 nm.

The absorbance was recorded. The IC_{50} values (concentration of tested compound required to inhibit cell proliferation by 50%) were determined from the dose-response inhibition curve.

3. Results

The extracted CV with chloroform (CVC) were tested to the normal cell (WRL68), breast cancer cell (MCF7) and human liver cancer cell lines (HepG2). The cytotoxicity of CVC with chloroform extract shown 50% inhibition (IC_{50}) of cells with 89 µg/ml after 72 hours period incubation for MCF7 compared to the HepG2. There slightly indicates that there was some inhibition did not reach (IC_{50}) on at 24 and 48 hour treatment period for both cancer cell lines. The HepG2 also showed the same effect after 72 hour treatment period (data shown at table 1). The ethanol extraction on CV (ECV) indicates that there was some inhibition at 24, 48 and 72 hour compared to the WRL68. The cytotoxicity assay of ECV extract on the HepG2 shown no effect on the experiment (data shown at table 2).

Table 1: Cytotoxic assay of CVC extract on MCF7, HepG2 and WRL68 at24, 48 and 72 treatment period.

Chloroform extract												
Cell lines	MCF7			WRL68			HepG2			WRL68		
Treatment periods (hours)	24	48	72	24	48	72	24	48	72	24	48	72
Effective concentration (µg/mL)			89									
Occurrence of IC_{50}	*	*	+	-	-	-	*	*	*	-	-	-

(+) indicates that there was 50% inhibition (IC_{50}) of cells
(-) indicates that there was no inhibition of cells
(*) indicates that there was some inhibition but did not reach (IC_{50})

Table 2: Cytotoxic assay of CVE extract on MCF7, HepG2 and WRL68 at24, 48 and 72 treatment period.

Ethanol extract												
Cell lines	MCF7			WRL68			HepG2			WRL68		
Treatment periods (hours)	24	48	72	24	48	72	24	48	72	24	48	72
Effective concentration (µg/mL)												
Occurrence of IC_{50}	*	*	*	-	-	-	-	-	-	-	-	-

(+) indicates that there was 50% inhibition (IC_{50}) of cells
(-) indicates that there was no inhibition of cells
(*) indicates that there was some inhibition but did not reach (IC_{50})

Whereas there was a positive effect of *Spirulina platensis* ethanol extract on MCF7 at 72 hour period with 85 µg/mL. There was indicates that there was some inhibition but did not reach (IC_{50}) at 24 and 48 hour (data shown at table 3). The Cytotoxic assay of *Spirulina platensis* ethanol (SPE) extract WRL68 indicates there was slightly inhibition at 48 and 72 hour but not reached 50% inhibition.

Table 3: Cytotoxic assay of SPE extract on MCF7 and WRL68 (24, 48 and 72 treatment period)

Cell lines	MCF7			WRL68		
Treatment periods (hours)	24	48	72	24	48	72
Effective concentration (µg/mL)			85			
Occurrence of IC_{50}	*	*	*	-	*	*

(+) indicates that there was 50% inhibition (IC_{50}) of cells
(-) indicates that there was no inhibition of cells
(*) indicates that there was some inhibition but did not reach (IC_{50})

4. Discussions

We demonstrated that locally grown CV and SP to be tested to the several cell lines such as MCF7,WRL68 and HepG2. CV extract inhibited the proliferation of MCF7 in a concentration dependent manner, ranging from 0-200 µg/ml as shown by cytotoxic assay. The HepG2 also showed the inhibition at 24, 48 and 72 treatment periods but not reached IC50 values. In others solvent such as ethanol extract were done to both microalgae and seemed to have performed better in inhibition (IC_{50}) of cells of human breast cancer (MCF7) rather than human liver cancer cell line (HepG2). The Cytotoxic assay of SPE extract shown the better result on breast cancer cells (MCF7) compared to the HepG2 with 85 µg/mL. Whereas, the *Chlorella vulgaris* ethanol (CVE) extract give the small effect on both MCF7 and no effect at all for HepG2 cell lines. The anticancer activities of purified SP were tested on growth and multiplication of human malignant melanoma cell line(A-375). The results indicate that Se-PC and PC significantly inhibit the proliferation of A-375 cells with IC50 values at 44.5 µM and 65.9 µM respectively (Chen *et a*l, 2006)

Most anticancer drugs exert their anticancer effects at the G1 or G2 stage to inhibit cell cycle progression (Harper JW *et al*., 1996). Although CV extract also inhibited the growth of WRL 68 cells, this however did not reached the IC50 in normal cells as shown in table 3. Our dose response experiments showed that CVC extract generated larger inhibition on cytotoxicity to the MCF7 with low doses such as 89 µg/mL compared to the HepG2 cell lines. Whereas for the SPE extract also generated the same effect at 85 µg/mL to reach the 50% inhibition (IC_{50}) of cells. The high concentration of CV and SP extract for both solvent must be conducted in the next experiment especially for the HepG2 which would be the subject of interest in our future study.

5. Conclusion.

CV and SP have been proven in various studies to display anticancer properties towards several kinds of cancers. It can be concluded from this study that microalgae has 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 caused by the extract or visual detection and confirmation of apoptosis.

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