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Antiproliferative activity of the *Chlorella* sp., SRD3 crude extracts against MCF-7 and Hep2 cell lines

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Abstract: **The main focus of the present study was to evaluate the in vitro cytotoxic effect of *Chlorella* sp., SRD3 agsinst cancer cell lines. The Microalgal solvent extracts showed cytotoxicity against breast cancer and Human cervix carcinoma cell line using the MTT reduction colorimetric assay. The four varting solvents extracts of *Chlorella* sp., viz., methanol, ethyl acetate, chloroform and hexane. From the results it is clear that maximum inhibition of 1.2 mg/ml was observed in all the extracts but the IC50 value varies for each extracts. Methanolic extracts had shown inhibition of 72 % of Hep2 cancer cell line and 77 % cell death against MCF-7 cell line compared to the other extracts. IC_{50} value of the methanolic extracts was found to be 327 and 323.3 µg/ml against Hep2 and MCF7 cell lines respectively. From the results its evident that the methanolic extracts were non toxic to the vero cell lines at 400 µg/ml, hence these extracts may serve as a better source in pharmaceutical industries in treatment of cancer cells.**

Keywords: **Microalgae, Cancer, MTT, Crude extracts, Inhibition, Viability**

I. INTRODUCTION

Microalgae are a novel source of sustainable natural products with various applications as pharmaceuticals [1,2] nutraceuticals and food supplements [3]. Nowadays, a particular interest is conducted to isolate microalgae from extreme environments such as hot springs as a good source of natural products for diverse biotechnological demand [4,5,6]. Cancer has become one of the most annihilating diseases globally with more than 10 million new cases annually. The compounds isolated from natural sources have been the source of most of the active components of medicines. One of the studies revealed that since 1994, 50 % of the drugs approved are originated from natural sources. The drugs approved cover a variety of therapeutic indications; most of these are related to cancer treatment [7]. There is always a need to search alternative sources with more effectiveness, more accessibility, and least side effects. Most cancers are thought to be a result of genetic mutations in the DNA of the cancerous cells and these mutations can be a result of inherited mutations, metabolism mutations, cigarette smoking, diet (red meat, fried foods), alcohol, radiation, environmental pollutants, and infectious organisms i-e viruses, stress, obesity, and physical inactivity. These causative elements might act in sequence or together to pledge or stimulate cancers [8,9,10]. Despite the current drugs present that manage to suppress the tumor growth, there is an urgent need to explore alternative strategies to overcome several limitations in treating breast cancer including the metastasis of cancerous cells which is the leading cause of mortality and morbidity, increasing the sensitivity of immune system response, and reducing the inflammation caused by cancer. With the advance of research to date, many medicinal plants have been subjected to scientific scrutiny where their secondary metabolites/bioactive compounds are discovered to have the anticancer effect potential. *Chlorella* sp., is reported to show

antioxidant activity Miranda et al, [11] in exhibiting attenuating effects on oxidative stress and suppressing inflammatory mediator activation in peritoneal macrophages and liver Lee et al., [12].However, there is little information about the effect of spirulina and chlorella on HSC and HepG2. It would therefore be useful to compare the antioxidant activity of spirulina and chlorella and to study their antiproliferative effects on HSC and HepG2 cells. There is always a need to search alternative sources with more effectiveness, more accessibility, and least side effects. The present study is focused on exploring the anticancer activity of extracts of the *Chlorella* sp., in cultured human breast cancer cells MCF-7 and Hep2 human cervix carcinoma.

II. MATERIALS AND METHODS

Microalgae strain

The microalga, *Chlorella* sp., SRD3 was obtained from applied microbiology laboratory and its growth was measured in terms of Optical density at 600 nm. This culture was grown in Bold basal medium and a temperature of 27 °C and pH 7.2 was maintained for its growth.

Chemicals

Neutral red solution, Fetal Bovine serum (FBS), Actinomycin- D, Dulbecco's modified Eagle medium (DMEM) and 4, 6-diamidineo-2-phenylinldole (DAPI), MTT (methyl-thiazolyltertrazolium), DMSO (dimthylsulphoxide) were obtained from Sigma Chemical Co. (St Lois, MO, USA). The drugs Gentamycin, Streptomycin, Glutamine, Actinomycin-D used in the experiments were also purchased from Sigma Chemical Co. (St Lois, MO, USA).

Preparation of the crude extract

The crude extract of the *Chlorella* sp., SRD3 were tested for cell cytotoxicity against Hep2 and MCF7 cancer cell line. A series of six dilutions (200, 400, 600, 800, 1000 and 1200 µg of final concentration) of crude extracts were prepared in DMEM (100 µl) containing DMSO (Dimethyl sulfoxide, maximum: 0.01%). Upon initial screening results, a test dose for MTT assay was set for extracts based on their apparent IC_{50} values.

Cell culture

The cell lines, MCF-7 and Hep2 were obtained from the American Type Culture Collection (ATCC). The MCF-7 and Hep2 were maintained in RPMI 1640 medium while MDA-MB-231 cell was maintained in DMEM medium. Both media were supplemented with 10 % Fetal Bovine Serum (FBS) and 1 % Penicillin/Streptomycin. MCF-7 on the other hand, was maintained in DMEMF12 medium supplemented with hydrocortisone (0.5 µg/ mL), insulin (10 µg/mL), hEGF (20 ng/mL) and 10 % FBS. The cells were grown in a humidified incubator at 37 °C in the presence of 5 % CO_2. The cell was passaged upon reaching 70 % confluency.

MTT (methyl-thiazolyltertrazolium) assay

After a 24 h exposure of test period, the determinations of toxic endpoints were carried out by methyl-thiazolyltertrazolium (MTT) assay. The quantification of cancer cells growth was carried out as described by [13,14]. After test period of 24 h exposure to extract, the cells were washed with phosphate buffer saline (PBS) twice. 10 µl of MTT reagent (5 mg/ ml in PBS) was poured to each well including the blanks (contained medium only). The plates were incubated for 4 h at 37 °C. Afterward, cells were washed with PBS twice, and 100 µl/well DMSO was added as a solvent to dissolve the insoluble crystalline formazan products. The effect of plant leaves, bark and fruit extracts on cancer cells was quantified as the percentage of control absorbance of reduced dye at 550 nm on a microplate reader (LabtechLT-4000MS, Labtechm International Ltd., East Sussex, and UK). Five replicates wells were examined for each treatment, and each experiment was repeated three times (n = 3) to calculate the standard error of mean. The results were calculated as percentage growth inhibition, untreated (control) cells versus treated cells according to the following formula:

% Growth Inhibition = Control− actual absorbance/ control × 100

Absorbance of the media was subtracted, both from control and treated cells.

Data presentation and statistical analysis

All data were compiled from a minimum of three experiments. Data for statistical analysis were expressed as 'standard error of the mean', n (number of experiments). The software used was GraphPad Prism version 6.00 for Windows, (GraphPad Software, San Diego California, USA).

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III. RESULTS

Cytotoxicity assay

The cytotoxicity of the vero cell lines was tested against the four extracts and it was found that 400 µg/ml of methanolic extract was not lethal to the vero cell lines . Chloroform extracts till the concentration of 200 µg/ml were not lethal to the vero cells. Whereas the other two extracts exhibited cell damage even at low concentration tested (Fig 1).

MTT assay

The various crude extracts of the microalga, *Chlorella* sp., SRD3, were subjected to MTT assay using two breast cancer cell lines to assess potential cytotoxicity. All the extracts showed a concentration dependent growth inhibition from 1000 to 1200 µg/ml of MCF-7 and Hep2 cancer cell lines. The effect of microalgal extracts against cell lines MCF-7 and Hep2 cell lines at a concentration of 100 µg/ml in MTT assays Compared to all the four extracts tested maximum death rate of 77.5 % in terms of inhibition was showed by methanolic extract with a concentration of 1200 µg/ml and IC50 value of 327 µg/ml was attained against Hep2 cell line. IC50 value of the other extracts such as ethyl acetate, Chloroform and hexane for Hep2 cell line was found to be 674.1, 742 and 865.9µg/ml. Whereas for the breast cancer cell lines the maximum inhibition of 79.5 % was observed at the concentration of 1200 µg/ml and IC50 value of 323.3 µg/ml was attained. The IC50 value of the other extracts viz., ethyl acetate, Chloroform and hexane for Hep2 against the MCF-7 cell line was found to be 621.6, 652.2 and 831.9 µg/ml (Fig 2, 3 and 4).

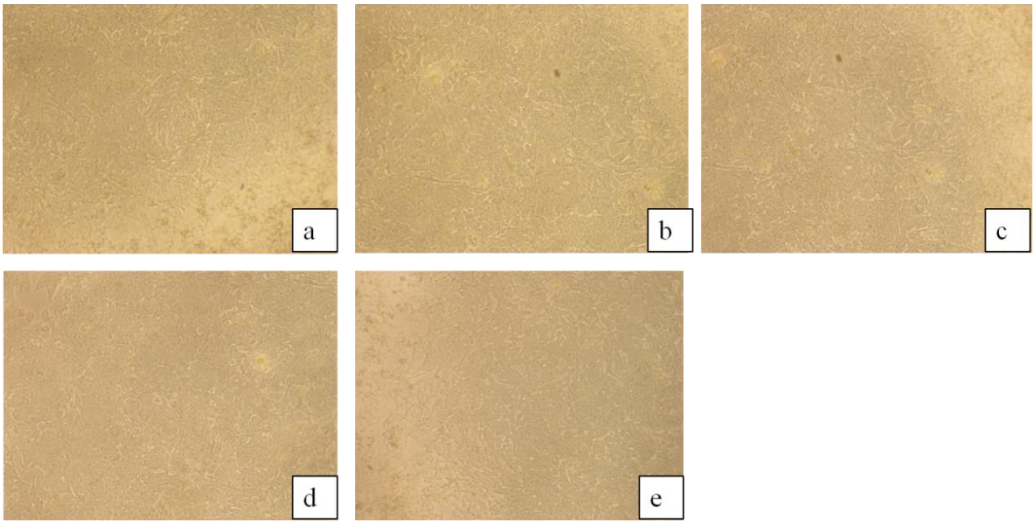

Fig 1. Cytotoxicity of the crude *Chlorella* sp., against Vero cell lines at 1000 µg/ml a) Control, b) Hexane, c) Chloroform, d) Ethyl acetate and e) Methanol

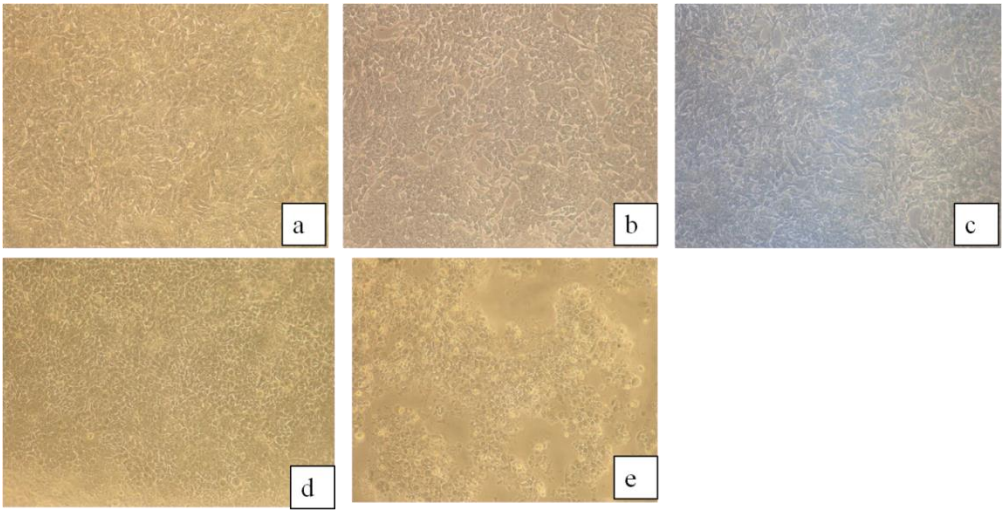

Fig 2. Anticancer activity of the crude *Chlorella* sp., against Hep2 cell lines at 1000 µg/ml a) Control, b) Hexane, c) Chloroform, d) Ethyl acetate and e) Methanol

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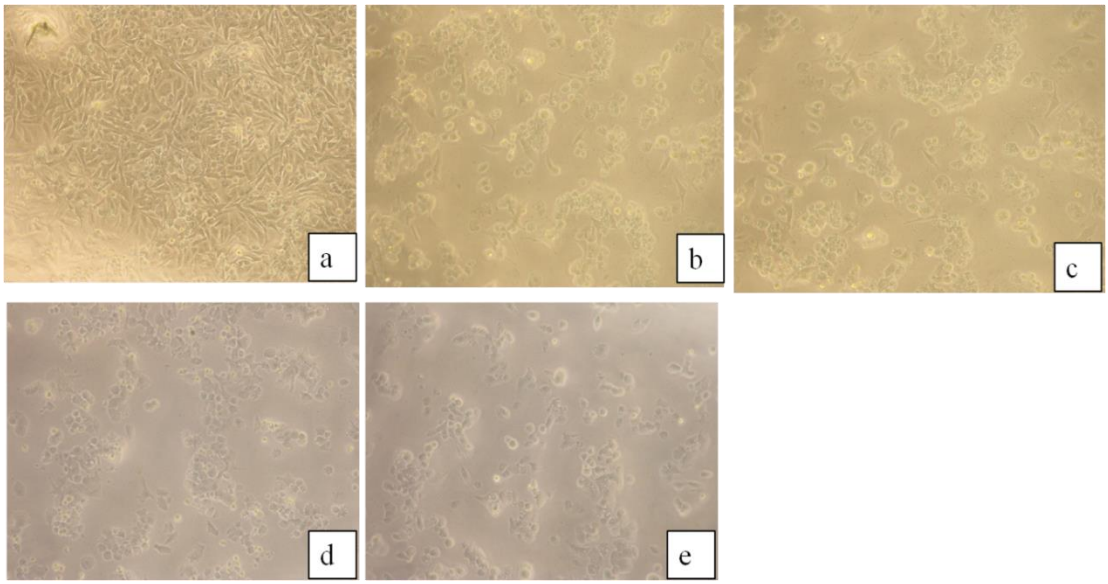

Fig 3. Anticancer activity of the crude *Chlorella* sp., against MCF-7 cell lines at 1000 µg/ml
a) Control, b) Hexane, c) Chloroform, d) Ethyl acetate and e) Methanol

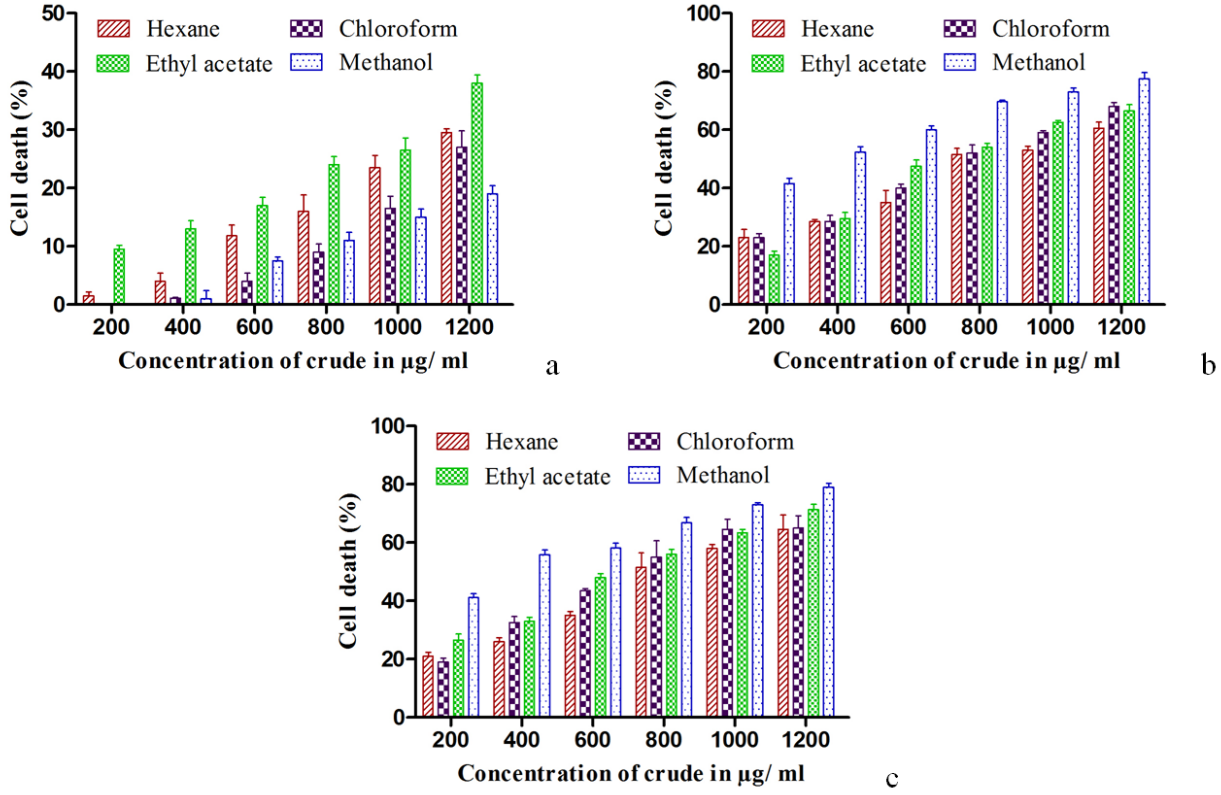

Fig 4. Effect of different concentration of *Chlorella* sp., SRD3 solvent extracts against cell lines p>0.05, n=2 a) Vero cell b) Human cervix carcinoma -Hep2 c) Breast cancer-MCF7

IV. DISCUSSSION

The present study focuses on evaluating the efficacy of varying crude extracts against breast cancer and Human laryngeal carcinoma- cell lines. Yusof et al., [15] reported the In vitro tumor cell toxicity assays resulted in concentration-dependent activity against the murine tumor cell line L5178Y-R (up to 61.9% and 74.8 % cytotoxicity at 500 µg/mL C. Sorokiniana and *Scenedesmus*sp. extracts, respectively). These results are comparable with other reports showing about 50% invitro cytotoxicity by microalga extracts against cervical cancer [16]. Apoptosis is the best known pathway for programmed cell

death. Apoptosis and necrosis can occur independently, sequentially or simultaneously. The type and/or the stimuli degree may determine if cells die by apoptosis or necrosis. At low doses, a variety of injurious stimuli such as heat, radiation, hypoxia, and cytotoxic anticancer drugs can induce apoptosis, or lead to necrosis at higher doses. After cells enter the apoptotic process, their DNA degrades, showing a ladder pattern of multiples of approximately 200 base pairs, which can be observed when extracting the DNA and making an agarose gel electrophoresis. Apoptosis involves the activation of caspases enzymes linked to the initiating stimuli. Caspase-3 is required for apoptosis-associated chromatin margination, DNA fragmentation, and nuclear collapse of the cell [17].

V. CONCLUSION

The present study focuses on testing the efficacy of the extracted *Chlorella* sp., against Hep2 and MCF-7. Methanolic extracts showed maximum inhibition against cancer cell lines at higher concentration. Based on the study it is clear that these chloroform extracts may serve as a better tool in pharma industries.

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