A NOVEL POTENT DRUG MOLECULES FROM CHLORELLA VULGARIS FOR THE CONTROL OF CANCER CELLS

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ABSTRACT

Algae have being investigated as a possible source of powerful pharmacological compounds to combat cancer cells. Following that, the MTT assay was used to assess the extracted compounds. Several chemicals were extracted and purified from C. vulgaris using chromatographic methods, and their structures were established using spectroscopic investigation. Using the MTT assay, these substances were then tested for their cytotoxicity against human cancer cell lines. The strongest chemical displayed selective cytotoxicity against cancer cells with low toxicity towards normal cells.The most active substance demonstrated little cytotoxicity towards normal cells and selective cytotoxicity towards cancer cells. The chemical caused cancer cells to undergo apoptosis by activating capsizes and up regulating pro-apoptotic proteins, according to mechanistic analyses.Through the activation of capsizes and the overexpression of pro-apoptotic proteins, mechanistic studies showed that the chemical caused apoptosis in cancer cells. In conclusion, our results point to C. vulgaris as a potential source of new pharmacological compounds with powerful anticancer characteristics, and more research is required to investigate their therapeutic potential.In our research, we evaluated that the cytotoxicity effect of a novel potent drug molecules from chlorella vulgaris.

Keywords: Algae;Chlorella;Bioactive compound; Cytotoxicity; Pharma activity

INTRODUCTION

Uncontrolled cell proliferation and cancer cells' capacity to penetrate healthy tissues make cancer a difficult disease. With millions of new cases being identified each year, it is one of the major causes of mortality in the globe. There is an urgent need for novel therapeutic choices since despite substantial advancements in cancer research; effective treatments for many different forms of cancer remain elusive. Several health advantages of chlorella vulgaris, freshwater green microalgae, have been demonstrated, including its capacity to fight cancer. Polysaccharides, peptides, and pigments are among the bioactive substances it contains that have been claimed

to have anticancer potential. In this study, we seek to locate novel, highly efficient therapeutic compounds from Chlorella vulgaris that are capable of successfully suppressing cancer cells. We will identify and characterize the bioactive components from Chlorella vulgaris using a variety of biochemical and biophysical approaches. Then, utilizing in vitro and in vivo experiments, we will assess these drugs' anticancer potential. The ultimate objective of this research is the creation of innovative medications from Chlorella vulgaris that are capable of efficiently treating different cancer types while having a low risk of adverse effects. This research may offer fresh perspectives on the ways in which Chlorella vulgaris works as well as point out potential new cancer-fighting medication candidates.

Among all causes of death, cancer is the second most common. Additionally, it was responsible for 8.8 million fatalities in 2015. Thus, cancer was the cause of almost 1 in 6 fatalities. Tumors that are cancerous, uncontrolled cellular Proliferations have a significant impact on the pathology.In addition to the more than 200 different types of malignant tumours that are described, other cancer types that can spread to other tissues and cause lethal metastatic tumours are also discussed. Due to this significant impact, cancer eradication has received a lot of attention(Jayaprakasam etal.,1958).Plants have been an essential source of conventional and clinically valuable drugs for the treatment of numerous forms of tumors (Cragg etal.,2005). An appealing method of developing anti-cancer drugs appears to be looking into plants for their potent anticancer properties with comparatively few side effects. Algae typically create a lot of natural anticancer amalgams or their metabolites (Sharif etal.,2014).Exopolysaccharide (EPS) is a linear or branched macromolecule that is secreted by microorganisms as slime or associated with cell surfaces. It has received a lot of research attention because of its environmentally friendly properties and applications, including biofloc, anti biofilm, antioxidant, and antitumor (Xiong etal., Shofia etal.,2018). Additionally, EPS may shield the cells from harmful substances such antibiotics, toxic metals, phagocytosis, and others (Kanmani, 2009;Ozturk, 2011, Abedini, 2018).The high value intracellular products of microalgae, such as lipid, protein, carotene, astaxanthin, lutein, and fucoxanthin, have attracted growing interest(Zhuetal.,2015; Overlandetal.,2019;Mathimani etal.,2019). These options make micro algal EPS another intriguing source of natural bioactive compounds. Micro algal EPS is far less understood than EPS from bacteria. EPS production and partial characterization from various algae species have only recently been reported in a small number of studies, including those on green algae (Dunaliellasalina, Chlorella vulgaris, Chlorella ellipsoidea, Chlorella pyrenoidosa, etc.) (Delattre et al., 2016), diatoms (Naviculasalinarum, Cymbellacistula, Pinnulariaviridis, etc.,)(Benvenuti et al., 2016).A lot of research has been done to examine the byproducts of micro algal metabolism, not only to understand their nature but also to find compounds with potential benefits for humans in a range of fields of interest (Furkert etal.,2006).

The objective of the current study is to assess a novel, potent drug's anticancer efficacy. Molecules from the chlorella vulgaris plant were used in the evaluation.

Materials:

In our research, we used several chemicals and strains to carry out the experiments and we purchased the chemicals, namely, Methanol, acetone, and distilled water,DPPH, Folin-Ciocalteu reagent, and RPMI-1640 medium, 10% FBS, 100 units/mLpenicillin, and 100 mg/mL streptomycin from the KrishnaramanChemicals Pvt.Ltd, Chennai,Tamilnadu.

Methodology:

Collectionofgreenalgae

The microalgae species of chlorella vulgaris was collected from Tamil Nadu lake water bodies.The algal sample was cleaned and other unwanted debris was removed. Then the sample wasrinsedwithsterilewater.The pure culture of the sample was incubated in water medium. Pure cultures were harvested andcollected by centrifuging at 10,000 rpm for 3 min. The collected micro algal pellets were driedunder shade and made into a coarse powder with mechanical grinder for further use. The algaldried powders were used for extraction by Extraction Method with chloroform solvents. The drypowdersofextractwereresuspendedinthesameorganicsolventsataconcentrationof100mg/mlforfurtherphytochemicalscreening.

PreliminaryPhytochemicalScreening

Many qualitative tests as prescribed by standard procedure were performed for the identification ofvariousphytoconstituentsaspertheHarborneprocedure.

Phytochemicals	Positive/Negative
Flavonoids	Positive
Tannin	Positive
Phenolic compounds	Positive

Terpenoids	Positive
Alkaloids	Negative
Cardiac glycosides	Positive
Saponins	Positive
Carbohydrates	Positive

Anticancer activityCelllines

CelllinesHepaticcarcinoma(HepG2cellline)wereusedinthisstudyandprocuredfromNationalCentreforCellSciences (NCCS),Pune,India.

Culturemedium

The liver cancer cell line HepG2 preserved in RPMI-1640 medium with 10% FBS, 100 units/mLpenicillin, and 100 mg/mL streptomycin in tissue culture flasks at 37 °C under a humidified 5 %CO_2and95%air.

Preparationof testsolutions

The chlorella vulgaris extract were separately dissolved in distilled water and the volumewas made up with medium supplemented with 2% inactivated FBS to obtain a stock solution of1mg/ml concentration andsterilizedby filtration. From this stock solution, five different lowerdilutions(100,200,300,400and500µg/ml)were prepared.

Cell Viabilityassay

MTTAssayisacolorimetricassaythatmeasuresthereductionofyellow3-(4,5dimethythiazol-2-yl)-2,5-diphenyltetrazoliumbromide(MTT)bymitochondrialsuccinatedehydrogenase.CancerHepG2cells wereseededatthedensityof2×10^5cells/wellwereplatedon6wellplatesandtreatedwithextractfor48h.Thecellswerepermittedtoadherefor24hours,andthegrowthmedium(MEM)removedusingmicropipetteandthemonolayerofcellswashedtwicewith MEM without FBS to remove dead cells and excess FBS. 1ml of medium (without FBS)containingdifferentdilutionoftestdrugswereaddedinrespectivewells;20µlofMTT(5mg/ml

InPBS)wereaddedtoeachwell,andthecellsincubatedforafurther6-7hrsin5%CO_2incubator.Afterremovalofthemedium,1mlofDMSOwasaddedtoeachwell.Theeffectofextractsoncellgrowth inhibition was assessed as percent cell viability, where vehicle-treated cells were taken asnegative control. The cells were then exposed to standard (as positive control). Concentrations ofthe chlorella vulgaris hydrolysate ranging 100-500 µg/ml and doxorubicin 100 µg/ml were usedfor the study. The supernatant was removed and 50 µl of propanol was added until the formazanproduct formed. The cells were incubated with the chlorella vulgaris hydrolysate for 48 h and thecell mortality were checked. The plates were read on an enzyme-linked immunosorbent assay(ELISA) reader at 570 nm. Each experiment was carried out in triplicate and the half maximalinhibitory concentration (IC_{50}) of this algal extract as the percentage survival of the cells wascalculatedaccordingtotheformulaprovidedbelow:

Percentageofviable cell concentrationwascalculatedthus:

$$\text{Viability}(\%) = (\text{MeanSampleOD}/\text{ControlOD}) \times 100$$

Statistics

Cell viability assay was measured as optical density at 570 nm. For statistical analysis ofdata,multiplecomparisonswereperformedusingone-wayanalysisofvariance(ANOVA)followed by the LSD test for post hoc analysis. Statistical significance was accepted at a level of$P<0.05$.DatawereanalyzedusingSPSS(version11).

RESULTS

S.No	Treatment	Conc(µg/ml)	Absorbance 570nm	%Cellviability
1	Negativecontrol		0.326±0.12	100±8.4
2	CVHtreated	100	0.286±0.10	87.7 ± 5.3^{*a}
3		200	0.253±0.09	77.6±6.1^{*a}
4		300	0.226±0.17	69.3 ± 4.4^{**a}
5		400	0.194±0.13	59.5 ± 5.1^{***a}
6		500	0.119±0.18	36.5±3.5***
7	Doxorubicin	100	0.085±0.06	26.0±1.8***

Valuesaremean±SEMexpressedas(n=3);
*P<0.05;**P<0.01;***P<0.001;^{a}P<0.001statisticallysignificantascomparedwithNegativecontrol.

CVH – Chlorella vulgaris hydrolysate, IC50 value is – 336.3 µg/ml

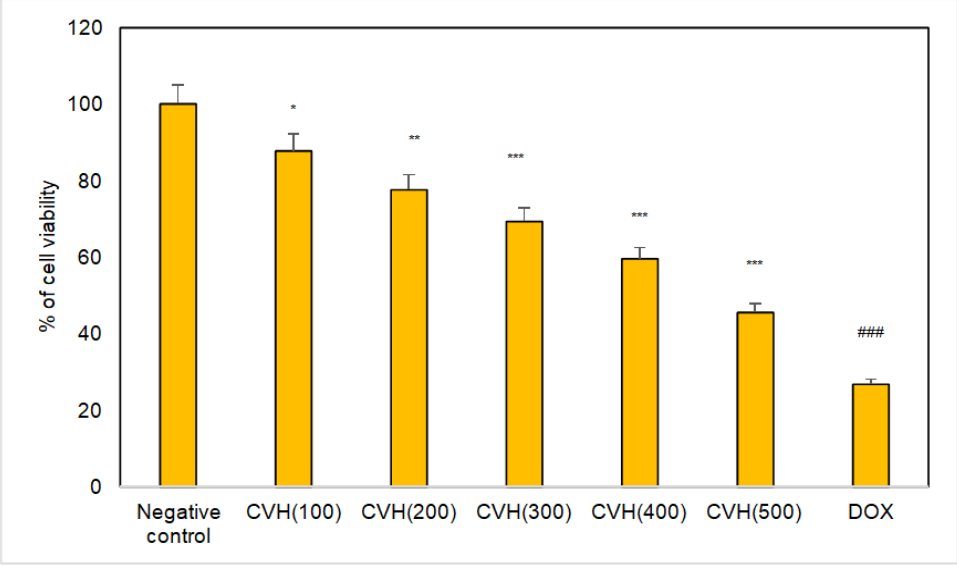

Fig1:ThecelldeathontheHepG2cellline.Valuesaremean±SEMexpressedas(n=3);

*P<0.05;**P<0.01;***P<0.001;$^{###}$P<0.001statisticallysignificantascomparedwith Negativecontrol.

AsshowninFigure1thepotentcellmortalityishigheratthe concentration500µg/ml.

Discussion:

A promising field of research involves finding new therapeutic compounds from natural sources, such as Chlorella vulgaris, to regulate cancer cells. Freshwater green alga Chlorella vulgaris has undergone substantial research for its possible health advantages, particularly its anticancer qualities. In Chlorella vulgaris, bioactive substances have been found to have anticancer activity, according to numerous researches. Chlorophylls, carotenoids, polysaccharides, and peptides are a few of these substances. For instance, it has been demonstrated that chlorophylls have antioxidant and anticancer properties by causing cancer cells to undergo cell cycle arrest and apoptosis. By altering the immune system and causing cancer cells to undergo apoptosis, polysaccharides from Chlorella vulgaris have also been proven to have anticancer potential. Additionally, it has been revealed that Chlorella vulgaris peptides have anticancer properties by preventing cancer cells from proliferating and migrating. A promising field of research involves the identification of new therapeutic compounds from Chlorella vulgaris for the management of cancer cells. To identify and characterize the bioactive substances found in Chlorella vulgaris and to ascertain their modes of action, additional research is nonetheless required. Additionally, considerable preclinical and clinical research will be necessary to transform these therapeutic compounds into medicines that are clinically effective in order to guarantee their safety. Despite these obstacles, the identification of novel therapeutic compounds from natural sources, such as Chlorella vulgaris, holds considerable promise for the creation of new, cost-effective, and safe cancer treatments.Hui-Min Wang *etal*., (2010) described that the active components (such as antioxidants) from a novel microalga, Chlorella vulgaris C-C, were extracted using supercritical carbon dioxide extraction (SC-CO2) technology because of its superior advantages over traditional solvent or ultrasonic extraction techniques. The polyphenol concentrations of C. vulgaris C-C were 13.40 and 0.46 (mg gallic acid/g lyophilized extract), respectively, using SC-

CO2 and ultrasonic extractions. In addition, SC-CO2 extraction yielded considerably more flavonoids (3.18 mg quercetin/g lyophilized extract) than ultrasonic extraction (0.86 mg quercetin/g lyophilized extract). Strong antioxidant activities in radical scavenging, ferric reducing power, and metal chelating abilities have been found in the C. vulgaris C-C extract from SC-CO2. The extract of C. vulgaris C-C suppresses the growth of human lung cancer H1299, A549, and H1437 cells in a dose-dependent manner in an experiment for cell proliferation. Eman A. El-fayoumy*etal.*,(2021), the authors advise large-scale Chlorella vulgaris production under a variety of stress circumstances for the use of the crude extracts and semi-purified fractions for creating a pharmacy-economic value in Egypt and other nations".

Ragaa A. Hamouda*etal.,* (2022) reported that In terms of antioxidant effects, thiamine was the best vitamin. Thiamine supplementation of C. vulgaris resulted in significant anticancer effects in vitro. Therefore, vitamins must be added to BG11 media to improve growth and metabolite production.YaserJafari*etal*., (2016) reviwed that the Cur loading was substantially higher in the microcapsules—up to around 55% w/w—than in other known bio-carriers. The research demonstrated that Chlorella vulgaris cell can serve as a new, stable delivery system for Cur.

Jianzhi Zhang *etal*., (2019) reviewed that the additionally, human colon cancer cell lines HCT8 were used in the research to examine the anticancer effects. According to the findings, they significantly reduced cell viability (28.3–18.0% on HCT8, respectively) and had antitumor effects. It is therefore important to continue researching these exopolysaccharides from microalgae species as a substitute for prospective antitumor agents.A suitable treatment plan is necessary for cancer, a common medical concern. the cell's malfunction,cycle is a recognized factor in the development of cancer. The main available therapeutic technique for treating cancer is chemotherapy and radiation; however, serious adverse effects have been associated with such actions.

Mohamed E. Abd El-Hacketal., (2018) explained about the radiation, for instance, has a negative impact on a patient's immune system's effectiveness.By offering new therapeutic solutions, these negative effects may be reduced. Compounds from fresh or marine flora, notably micro- and macroalgae, have been documented to have complementary and alternative therapeutic properties that have anti-cancerous properties. Microalgae also contain a variety of bioactive

molecules, such as carotenoids, different kinds of polysaccharides, vitamins, sterol, fibres, minerals, etc. The large amount of untapped biomass in microalgae and their outstanding diversity of chemical constituents may represent a significant advancement in the creation of anti-malignant drugs. In the past, this trait of micro algal biodiversity was used for profit to produce dietary supplements and gelling agents. However, recently, a number of studies were conducted to examine the possible anti-carcinogenic activity of micro algal extracts, and they largely concluded that these extracts have the ability to cause the apoptotic death of cancer cells through caspase dependent or independent routes. In this review work, we described the various micro algal species that had anti-tumor properties, the tumor cell lines that were changed by the use of micro algal extracts, and the concentrations of these extracts that were effective.

Conclusion:

In recent years, discovering new drugs has mostly been fueled by natural products, particularly those made from microalgae like Chlorella vulgaris. These organisms generate a wide variety of secondary metabolites with distinctive structures and bioactivities that may be used to create fresh treatments. The bioactive components of Chlorella vulgaris and its potential anticancer action have been the subject of numerous investigations. Studies have demonstrated, for instance, that Chlorella vulgaris extract and its components—such as chlorophyll, carotenoids, and polysaccharides—display anticancer potential against a variety of cancer cell lines in vitro. However, creating a therapeutic molecule from natural sources is a difficult procedure that necessitates meticulous scientific validation. This process includes locating the active ingredients, evaluating their pharmacological characteristics, and doing clinical studies to check for safety and efficacy. It is also important to remember that creating a therapeutic molecule is an expensive and time-consuming operation. In conclusion, although the development of drug molecules from Chlorella vulgaris is promising, more study is required to confirm the anticancer activities of its bioactive components and to turn them into secure and efficient medicines for the treatment of cancer.In our research study we identified that thepotentcellmortalitywashigheratthe concentration500µg/ml.Potent bioactive molecules in the fresh water green algal species chlorella vulgaris showed cytotoxicity effect against the liver cancer cell line hepg2.It showed pharmalogical activity against cancer cells,

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