Journal of Applied and Natural Science
16(1), 325 - 333 (2024)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Anti-oxidant and cytotoxic activity of *Spirulina platensis* ethanolic extract against Caco-2 and HepG2 cancer cell lines

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Article Info
https://doi.org/10.31018/jans.v16i1.5280
Received: November 20, 2023
Revised: February 17, 2024
Accepted: February 26, 2024

How to Cite
Al-saily, H.M.N. *et al.* (2024). Anti-oxidant and cytotoxic activity of *Spirulina platensis* ethanolic extract against Caco-2 and HepG2 cancer cell lines. *Journal of Applied and Natural Science*, 16(1), 325 - 333. https://doi.org/10.31018/jans.v16i1.5280

Abstract
Spirulina platensis is blue-green algae received significant attention for its high nutritional value, it is a source of powerful anti-oxidants. The cytotoxicity of crude extract is not well recorded. The aim of current study to evaluate the cytotoxicity of *S. platensis* extracts on colon cancer (CaCo-2), hepatic cancer (HepG2) cell lines, normal fibroblast cells line (HdFn) and also antioxidant activity. The percent of 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity was determined for serial concentrations of extract ranging from 3.125 to 200 µg/ml. Cell lines were treated for 24 hours with different concentrations of extract ranging from 25 to 400 µg/ml. Cell viability testing using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay which determined how the extract affected caspase 9 activity. The results revealed that extract had moderate antioxidant activity, showing the DPPH scavenging activity reached 58% in a concentration of 200µg/ml, and IC50 was 95.84 µg/ml. The extract significantly decreased CaCo-2 cell viability with IC50 99.12 µg/ml, compared to HdFn viability with IC50 157.6 µg/ml. On CaCo-2 cells, the extract's cytotoxicity was more evident ($P< 0.05$) than HdFn cells. The extract had more significant ($P<0.05$) cytotoxicity on cancer cell lines and also significantly decreased the viability of HepG2 cells with IC50 167.4 µg/ml, than the viability of HdFn with IC50 214.9 µg/ml. The extract revealed significantly higher ($P< 0.05$) cytotoxicity against HepG2 cells than the normal HdFn cells. This study concluded that the extract exerted a dose-dependent anti-proliferation effect on CaCo-2 cells and HepG2 cells by comparing them with HdFn cells.

Keywords: Anticancer agent, Antioxidant, cancer cells Ethanolic extract, *Spirulina platensis*

INTRODUCTION

One of the major methods which used to treat the cancer is chemotherapy since it kills or slows the cancer cells proliferation. The gravest diseases which threaten the health of human worldwide is cancer. In addition, this class of medicines has a negative reputation for being poisonous and unpleasant, with the potential to be fatal. In general, chemotherapy is used to treat advanced malignancies, whereas surgical excision is the primary line of treatment for early tumors. Although cancer treatment has advanced significantly thanks to current research, the effectiveness of chemotherapy medications remains restricted by drug-induced side effects and multidrug resistance (Anand *et al*., 2022). Traditional chemotherapy drugs frequently cause immune or myelo suppression (decrease of blood cells production), mucositis (inflammation the lining layer of digestive canal), and also alopecia (hair loss) challenges, which deteriorate the life quality of cancer patients. (Schirrmacher, 2019).

Marine resources, particularly seaweeds and microalgae which are considered a source of bioactive compound, are becoming more and more popular. (Garcia-Vaquero,2023). The qualities of spirulina (Arthrospira) as a possible pharmaceutical source of medicines have attracted the interest of the scientific and medical fields. It has been rightly termed "Super Food" or "Miracle from the Sea" by the FDA and WHO. (Gentscheva *et al*., 2023). Spirulina has become an increasingly common nutritional supplement and includes a variety of bioactive compounds. It is a unique mixture of various substances with medicinal value. Its different antioxidant components have a part in avoiding carcinogene-

sis (Kumar et al., 2022). *Spirulina* is a blue-green filamentous alga or cyanobacterium that is unbranched, helicoidal, and family Oscillatoriaceae (Ge *et al.,*2019). It naturally increases in a variety of aquatic environments factors, including saline, fresh, and alkaline waters, even in those with high values in pH (Tzachor *et al*., 2022).

Spirulina powder's approximate compositions are as follows: proteins (58.94%), carbohydrates (16.68%), fats (1.54%), ash (12.22%), fibers (1.0%), mineral salts of calcium (0.30%), phosphorus (1.08%), and total salt (1.17%), sand and silica (0.31%), moisture (9.62%), and gross energy (4183 kcal/kg), (Thangaraj *et al*.,2022). The oxidative effects of reactive oxygen species on lipids, proteins, and nucleic acids trigger chronic illnesses. Since certain synthetic antioxidants are harmful and carcinogenic in animal studies, natural antioxidants must be used in their place. Therefore, finding new sources of natural antioxidants that are both secure and affordable is crucial (Kumar *et al*., 2022). *Spirulina platensis* is highly sought-after by microalgae as a key source of proteins, carbohydrates, pigments, and antioxidants as well as a feedstock for biofuels (Panaite *et al*., 2023). *Spirulina* is an all-around health enhancer. Because it contains natural colors including alpha-carotene, chlorophyll, xanthophylls, phycoerythrin, and phycocyanin, it has the ability to scavenge free radicals. They might operate alone or together. Phycocyanin, and tetrapyrrolic chemicals that gives blue-green color to spirulina also has antioxidant properties, can protect against oxidative damage (Kumar *et al*., 2022).

Although few studies have been performed about the Spirulina benefits to the health, the evidence for its therapeutic potential applications is still not well clarified in the areas of antioxidant, and anticancer activities.The present study assessed the antioxidant and cytotoxic effects of Spirulina(*Spirulina platensis*) ethanolic extract in the growth of colon cell lines like CaCo-2 and hepatic cell lines hepG-2 as compared with normal cells represented by fibroblast cell line HdFn.

MATERIALS AND METHODS

Ethical approval

The study was conducted according to the ethical principles originating in the Declaration of Helsinki. It was carried out with patients' verbal and written approval before the sample was taken. The study protocol subject information, and consent from were reviewed and approved by University of Babylon ethical committee according to document number Z230506 dated 08-05-2023.

Preparation of algal extract

Spirulina platensis was obtained from Xi'an Daruidu Network Technology Co., Ltd (China) as a 100% pure Natural green spirulina powder . The powdered algal materials were extracted with solvent ethanol-water (70%) according to Ekpenyong *et al* (2012), using a few changes, one gram of algae powder, shaking 10 ml of solvent for an hour at room temperature. The suspension was placed in a water bath (30-37°C) for 30 minutes, filtered using gauze, and then concentrated to dryness in an oven at 45°C. The dried concentrated material was milled by using the electronic mill, and the final powder was sterilized by Ultraviolet (UV) equipment for 20 min.

Phytochemical analysis tests for ethanolic -watery extract of *Spirulina*

The alkaloids were screened using Dragendroffs test; Mayer test and Wagner test following Qaisar *et al.* (2009). The Phenols were screend following (Deepa and Padmaja, 2012).The Flavonoids were detected according to (Bandarnayake, 2002). The Tannins and Carbohydrates using Molische test for the presence of carbohydrates were screened following (Qaisar *et al*., 2009). The Coumarins and Glycosides were detected following Patra *et al. (*2009). The Saponin was screened using Foam test and the Terpenoids were detected using Liebermann-Burchard test following Bandarnayake (2002). The Carboxylic acid was detected according to Singh and Kumar (2017). The Cardiac glycosides were screened following Rahman and Ahmed (2013). The phytosterols were detected according to (Kumar and Jat, 2018). The Quinons were screened following Basumatary, 2016). The Proteins and amino acids were screened following (Silva *et al*., 2017). The Anthocyanins were detected following (Obouayeba *et al*. (2015). The Gums and mucilages were detected following Raaman (2006).

DPPH radical scavenging method to evaluated the antioxidant activity

This procedure was following Kumar (2008). Briefly, 0.1 mM DPPH solution in ethanol was prepared. Three milliliters of various ethanolic extracte, each at different concentrations (200, 100, 50, 25, 12.5, 6.25, and 3.12 g/ml), were combined with one ml of this solution. The mixture was shaken briskly before let it stand for 30 minutes at room temperature. Then, the absorbance was detected at 517 nm by using spectrophotometer. The reference standard chemical which utilized was ascorbic acid, and a triplicate of the test was carried out. The IC 50 value of sample, or the quantity of the samples which required to inhibit 50% of DPPH free radical. 50 %, was calculated from the plotted graph of scavenging activity against the various concentrations. The % DPPH scavenging effect was calculated by using following equation:

DPPH scavenging action or inhibition percent

$$(\%) = \frac{A_0 - A_1}{A_0} \times 100$$ Eq. 1

A_1 the absorbance of the test or standard sample
A_0 the absorbance of the control.

Determination of cytotoxic activity of *Spirulina platensis* ethanolic extract

In *vitro* procedure was used to examine the potential cytotoxic impact of *S. platensis* ethanolic extract on cancer cell lines (CaCo-2) and (HepG-2) was compared with a normal cell line (HdFn). The cell lines were procured from the Pharmacology Department/ Medicine College/Malaya University. The cytotoxic effect of the *S.platensis* ethanolic extract was examined according to the indicators, such as the MTT test, which measures cell viability and Caspase 9, which measures cell apoptosis. For the Caspase 9 test, only the most cytotoxic concentration of the ethanolic extract and the most susceptible cells determined by the IC50 value of the most sensitve cells assessed by MTT were selected.

(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay

In order to detect the activity of cells metabolizms by Spectrophotometer, the MTT test technique was used that relies on the fact that the MTT can be reduced by metabolically active cells via succinate dehydrogenase mitochondrial enzyme to generate insoluble crystals of purple formazan that were afterwards soluble. Promega's Cell Titer 96® Non-Radioactive Cell Proliferation Assay was used for this investigation.

For the colon cancer cell line (CaCo-2), liver cancer cell line (HepG2) and type of normal fibroblast cell line (HdFn), 100 µl of cell suspension and two-fold serial dilutions of ethanolic extract were applied to 96 flat-bottomed microtiter plate wells, each line in a separate plate following the addition of 25, 50, 100, 200, and 400 µg/ml of S. platensis ethanolic extract, each well received a final volume of 200 µl of complete culture media. The plates were then incubated for 24 hours at 37° C with 5% CO2 using triplicates of each concentration and the controls, which were cells treated with serum-free medium. Each well received a 10 µl application of the MTT solution. 200 µl of the DMSO solubilization solution was added to each well. The plates were shaken for 5 minutes after the solution turned yellow after four hours of incubation at 37°C and 5% CO2. The DMSO mixture turned purple. The absorbance was measured using an ELISA reader after the dye had completely dissolved (Chih *et al*., 2004).

$$Cell\ viability\ perecent = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ untreated\ cells} \times 100$$ Eq.2

The optical density data were statistically analyzed to determine the concentration of extract needed to reduce cell viability by 50% for each cell line. (Freshney, 2012).

Detection of Caspase 9 in the CaCo-2 Cell Line

The Caspase-Glo®9 Assay recognizes the activity of caspase-9, which is essential for the intrinsic pathway of apoptosis in mammalian cells (Costantini *et al*., 2002). On a basic level, cells were seeded on white 96-well plates at a density of 1 x 104 cells per well in a 100 L final volume of complete culture media for each well. The plates were incubated for 24 hours at 37°C and 5% CO2. Following incubation, the medium was taken out, and 100 mL of Spirulina extract in 25 and 50 g/mL concentrations, were applied to the wells. As well as the negative control (cells treated with serum- free media and Dimethyl sulfoxide (DMSO) and the positive control (cells treated with serum-free medium and Tamx), triplicates were employed for each concentration. For 24 hours, plates were incubated at 37°C and 5 %CO_2. The plates were left to equilibrate at room temperature after incubation. Each well received aliquots of 100L of Caspase-Glo® 9 Reagent. Using a plate shaker, the plate was gently shaken for 0.5-2 minutes at 300-500 rpm. The plate was incubated at room temperature for 30 minutes to 3 hours, each sample's luminescence was quantified at 405 nm using an ELISA reader.

Statistical analysis

Analytical statistics were made to determine whether group variance was statistically significant. A one-way analysis of variance (ANOVA) (Duncan) test was used. Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla, California, USA, www.graphpad.com) was used to perform statistical significance tests on the data, which were reported as mean standard deviation.

RESULTS AND DISCUSSION

The 70% ethanolic extract of *S. platensis* used in this study was subjected to preliminary qualitative chemical testing, which verified the existence of flavonoids, phenols, alkaloids, terpenes, tannins, cardiac glycosides, saponins, coumarins, and phytosterols. However, this extract lacked carbohydrates, glycosides, quinones, proteins, amino acids, anthocyanins, gums, and mucilages (Table 1). These phytochemicals in this algae species may indicate that it may have some medicinal potential. These results agree with other studies which showed that the chemical analysis of a methanolic extract of *S. platensis* contains phenols, tannins, alkaloids, flavonoid, proteins and aminoacid and glycosides (Fayyad *et al*.,2019). The findings of several studies (Kumar *et al*., 2022) that detect the same chemicals in *Spirulina platensis* are supported by this observation. One of the most well-known phytochemicals with a variety of biological functions in safe quantities was flavo-

Table 1. Detection of some phytochemical compounds in ethanolic extract of *Spirulina platensis* by chemical tests

S.No.	Phytochemical Constituents	Test name	Result	Indication of reaction
1.	Alkaloids	Wagner's test	+	Reddish precipitate
		Mayer's test	+	white precipitate
		Picric	+	An orange coloure
		Iodine	+	A blue colour, which disappears on boiling andreappears on cooling
		Tannic acid (10%)	+	Buff colour precipitate
2.	Carbohydrates	Molish's	-	A violet ring
3.	Phenols	Drops of Ferric chloride	+	Dark green/bluish black
4.	Tannins	Ferric chloride alcoholic	+	Blue-green colour
5.	Flavonoids	Lead acetate test 10%	+	precipitate
6.	Saponins	Foam	+	1 cm layer of form
7.	Glycosides	Benedict reagent	+	brick red precipitate
8.	Terpenoids	Liebermann-Burchard test	+	A grey colour
9.	Coumarins	10% NaOH +chloroform	+	A yellow colour
10.	Carboxylic acid	Sodium bicarbonate	-	Appearance ofEffervescence
11.	Cardiac glycosides	Drops of Baljet test	+	A yellow-orange colour
12.	Phytosterols	Chloroform+H2SO4	+	Pink ring / Red colour
13.	Saponins	Foam	+	1 cm of foam
1 4.	Quinons	HCl	_	Green colour
15.	Proteins and amino Acids	Drops of nitric acid	+	Yellow colour
16.	Anthocyanins	HCl (2 N)+few ammonia	_	Pink-red
17.	Gums and Mucilages	Absolute alcoholstirring	_	White precipitate

noids.Terpenoids have several medicinal benefits (Gouda *et al.*, 2021). The other compounds which found in the extract and described as phytochemicals with pharmacological effects were saponins and alkaloids, (Alghamdi *et al.,* 2023).

Antioxidant activity of *S. platensis* ethanolic extract free radical scavenging activity of *S. platensis* ethanolic extract was studied using a stable DPPH free Radical scavenging assay. The results (Table 2) revealed that 200 µg/ml of *S. platensis* extract had 57.57% scavenging activity, while 100 µg/ml of extract had 52.17% scavenging activity and the concentration 50 µg/ml of extract had 40.84% scavenging activity. The results also revealed that 25 µg/ml of extract had 30.05% scavenging activity and the concentration 12.5 µg/ml of extract had 28.8% scavenging activity, while 6.25µg/ml of extract had 25% scavenging activity and the concentration 3.12 µg/ml of extract had 20.12% scavenging activity. Moreover, the present findings demonstrated that the ethanolic extract of *S. platensis* was dose-dependent in its ability to scavenge DPPH free radicals. When the extract's IC50 was computed, it was observed that the extract had an inhibitory effect on DPPH of 95.84 g/ml as shown in Fig. 1.

These results appeared that the extract of *S. platensis* possessed antioxidant activity in high concentrations. This matches with results of (El-Chaghaby *et al.,*2019) who found the highest total antioxidant activity in *S.platensis*. The result may be due to high phenolic contents in *S. platensis* (Ghobashy *et al*., 2022). Several phenol groups are present in Phenolic compounds, which donate a hydrogen atom or an electron making them very responsive in counteracting free radicals (Saffaryazdi *et al*., 2020). *Spirulina* also contains tocopherols and beta-carotene which exhibit antioxidant properties (Miranda *et al*., 1998).

Table 2. Percentage of scavenging activity of *Spirulina platensis* ethanolic extract.

Plant extract Concentration (µg/ml)	Scavenging activity (%)
200	57.57
100	52.17
50	40.84
25	30.05
12.5	28.8
6.25	25
3.12	20.12

MTT assay test for the cytotoxic effects of *S. platensis* ethanolic extract in vitro

The results on toxicity of *S. platensis,* of different concentrations of ethanolic extract (400, 200, 100, 50, and 25 µg/ml) used by MTT assay on two types of cancer cell lines: colon cancer cell line (CaCo-2), liver cancer

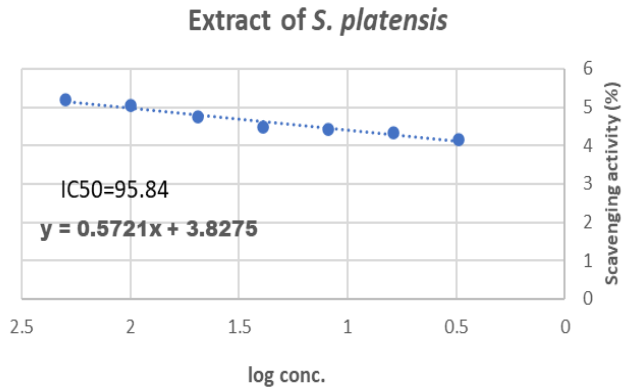

Fig. 1. *IC50 of Spirulina platensis extract*

cell line (HepG2) and one type of normal human fibroblast cell line (HdFn) are shown in (Table 3) ; and Fig. 3 and 4 respectively.

Table 3 revealed that *S. platensis* extract significantly decreased the viability of CaCo-2 cells , it was 29.39 %, 38.23 %, 51.08 %, 64.93 % and 73.34 % for 400, 200 ,100,50 and 25 µg/ml, respectively with IC50 99.12 µg/ml for all these concentrations. When compared with the viability of HdFn, it was 66.39% 74.76 %, 86.42%, 92.59 %, and 95.216 % for 400, 200,100 , 50 and 25 µg/ml, respectively, with IC50 157.6 for all these concentrations. When compared to the normal cell line HdFn, the extract's cytotoxic action on the cancer cell line CaCo-2 was more significant (P <0.05), as elucidated in (Fig. 3). The results shown in Table 3 and Fig.2 also revealed that *S. platensis* extract significantly decreased the viability of HepG2 cells, it was 41.62%, 50.84%, 62.73%, 68.98% and 73.26%, for 400, 200, 100, 50 and 25 µg/ml respectively with IC50 167.4µg/ml for all these concentrations when compared with viability of HdFn, it were 75.15%, 87.26%, 94.02%, 94.29% and 93.8 for 400,200,100,50, and 25 µg/ml respectively with IC50 214.9 µg/ml. In comparison to the normal cell line HdFn, the extract's cytotoxic action on the cancer cell line HepG2 was more substantial (P<0.05), as seen in (Fig.3).

These findings indicated that after incubating these cells with extract at various concentrations for 24 hours, the viability of the cells was reduced in a dose-dependent manner, with cell viability decreasing as the concentrations were raised., The maximum effect was at the concentration 400 µg\ml. The cancer cells were affected by extract more than normal cells, and the colon cancer cells CaCo-2 were more affected than liver cancer cells HepG2. These results agree with the results of Czerwonka *et al*. (2018), who showed that the Spirulina extract impacted the lung cancer cell line A549, which was cytotoxic and anti-proliferative. Spirulina also demonstrated cytotoxicity against hepatocellular carcinoma HEPG2 and colon cancer HCT116 cell lines (Zaid *et al*.,2015). The present results match the results of Fayyad *et al*. (2019) who revealed a dose- and time-dependent inhibitory impact on the cytotoxic activity of methanol extracts of spirulina against the two tested human cell lines. The methanol extracts of spirulina potentially inhibited the growth of L20B and MCF7 as compared to the control cells.

The phenols, tannins, alkaloids, and flavonoids that are previously reported as components of the extract may be to blame for the cytotoxicity that Spirulina extract displayed to cancer cell lines. Flavonoids are among the most well-known compounds with various biological activities at nontoxic concentrations. The potential of flavonoids as anticancer drugs has received considerable attention. These chemical groups are also known to have a variety of effects on tumor cells, including the prevention of cell growth, the induction of apoptosis, and the inhibition of kinase enzymes (Kopustinskiene *et al*., 2020) On cancer cells, flavonoids have a variety of actions, including the inactivation of the carcinogen, antiproliferation, and cell cycle arrest (Kapoor *et al*., 2021). Terpenoids have many medicinal qualities, including anticancer, antiallergenic, antiparasitic, anti-inflammatory, and immunomodulatory actions (Kamran *et al*., 2022). The additional compounds included in the extract like saponins and alkaloids also said to have an anticancer impact. In human colon cancer cells and tumor xenograft, saponins have been shown to inhibit proliferation and induce apoptosis (Dhyani *et al*., 2022). The reason is that cancer cells are affected by *S.*

Table 3. Viability of CO-2, $HepG_2$ and HdFn cell lines at different concentrations of *Spirulina platensis* extract after incubation for 24 hour at 37 C° in 5% CO2 (* P≤0.05)

		Viability%						
Concentrations (µg/ml)		**400**	**200**	**100**	**50**	**25**	**IC50**	**P value**
S. platensis Extract	CaCo-2	29.398 ±2.31	38.233± 0.94	51.08 ±3.084	64.93 ±1.36	73.341 ±1.27	99.12	<0.0001
	HdFn	66.39 ±1.52	74.768± 1.3	86.42 ±1.91	92.59 ±2.14	95.216 ±0.57	157.6	
S. platensis extract	HepG2	41.628 ±1.39	50.84 ±2.27	62.73 ±2.53	68.98 ±7.6	73.264 ±2.04	167.4	<0.0001
	HdFn	75.15 ±1.75	87.26 ±2.42	94.02 ±1.03	94.29 ±0.46	93.8 ±0.77	214.9	

* P≤0.05.

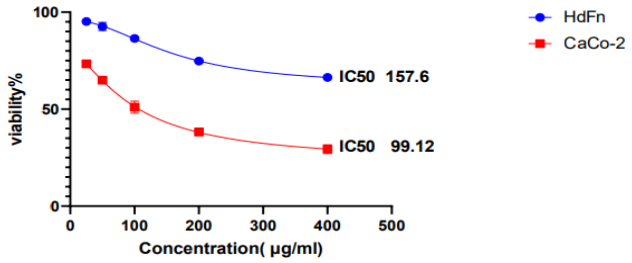

Fig. 2. *After 24 hes.of incubation, Spirulina platensis extract had acytotoxic impact on CaCo-2 and HdFn cells.*

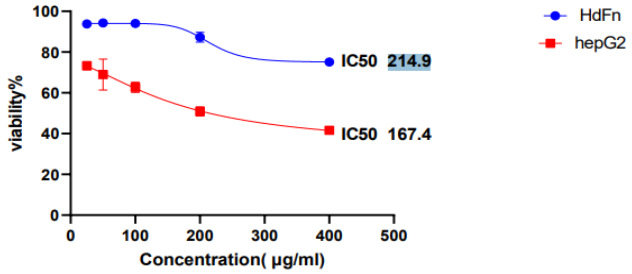

Fig. 3. *Cytotoxicity effect of Spirulina platensis extract on HepG2and HdFn cells after 24 hour of incubation*

platensis more than normal cells may be due to the slow rate of normal cells, whereas cancer cells have rapid cell division (Han *et al.,*2021). A slow rate of repair in cells which rapidly divide more critical than the slowly dividing cells with the same repair rate (Gao *et al.,*2021). In cancer cells, the number of DNA antimetabolites and enzyme inhibitors, which inhibit enzymes involved in DNA synthesis or its precursors, was increased, causing characteristic lesions in cancer cells which may increase their sensitivity to the toxicity of substances than in normal cells as toxicity scatter in this cells (Farag *et al.,* 2021). The nature of the chemicals in crude extract and their interactions with the metabolic characteristics of each kind of cancer cell or the efficiency of particular enzymes that serve as antioxidants specifically to cancer cells may be the causes of why colon cells are more impacted than liver cells (Roozi *et al.,* 2021). The reduction in growth of normal fibroblast cell lines, even at the highest concentrations, was less than cancer cell lines. This could further indicate the relative safety of crude extracts of *S. platensis* towards normal cells. Caspase 9 activity of CaCo-2 cells treated by *S. platensis* extract.

Table 4 and Fig. 5 revealed that extract and tamoxifen 20 µM (Substance used as cancer-preventing which indicates an effective control) significantly increased ($p<0.05$) the caspase 9 activity of CaCo-2 cell line

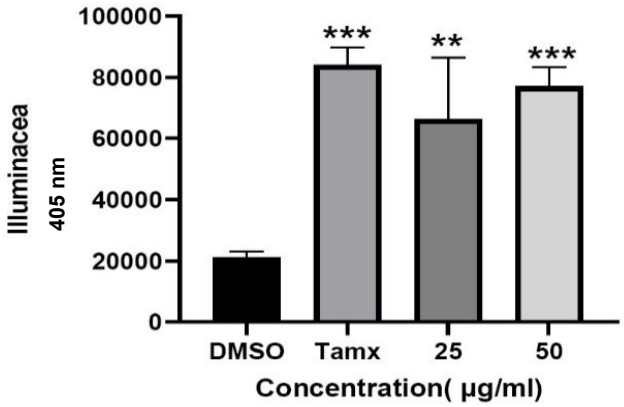

Fig. 4. *Caspase 9 activity of CaCo-2 treated with ethanolic extract of Spirulina platensis comparison with negative control (DMSO treated CaCo-2 without extract) and positive control (tamoxifen 20 µM)*

when compared with negative control (DMSO). Caspase 9 activity of CaCo-2 treated with *S. platensis* extract in 50 and 25 µg/ml was significantly increased. It was 77078.6 and 66339.3, respectively, compared to the negative control. Caspase 9 is part of "cysteine dependent aspartate-specific proteases" (caspase family), which play a pronounced role in apoptosis (Plassmeyer *et al.,* 2021). It is activated during the intrinsic pathway of apoptosis (Madadi *et al.,* 2021).

The results indicated that CaC-2 treated with *S. platensis* extract undergoes intrinsic apoptosis through increased caspases 9 activity and cytochrome C released from mitochondria (Kale *et al*., 2018). Many kinds of damage or stress signals (DNA damage, cell cycle checkpoint defects and hypoxia) may converge at the mitochondrial outer membrane of CaCo-2 treated with *S. platensis* extract where cell fate is ultimately decided (Kerkhofs *et al*.,2021). In response to these signals, CaCo-2 cells activate BCL-2 (B cell lymphoma 2) household protein, which represents those who control the mitochondrial apoptotic pathway most effectively; these proteins are encoded in humans by the BCL2 gene (Gao *et al*.,2021). BCL-2 proteins are divided into three groups: anti-apoptotic proteins (such as BCL-2 and BCL-XL), which preserve mitochondrial integrity, pro-apoptotic proteins such as BAX and BAK are essential effectors for mitochondrial outer membrane permeability and BH3-only proteins such as BID and BAD that promote apoptosis by activating the pro-apoptotic proteins or inactivating the anti-apoptotic proteins. In response to these damages or stresses signals, activated BH3-only proteins lead to (BAK) and (BAX) proteins

Table 4. Caspase 9 activity induction in CaCo-2 treated cells with ethanolic extract of *Spirulina platensis*.

Concentrations (µg/mL)	Caspase 9 activity (mean ± SD)	Significant	P-value
Negative control (DMSO)	21124.66±1960.19	----------	---------
positive control (Tamx.)	84119.66±5707.35	***	0.0001
50 µg/mL	77078.6± 6190.60	***	0.0001
25 µg/mL	66339.3± 20049.04	**	0.0001

activation (Huang *et al*., 2021). Mitochondrial outer membrane permeability induced by BAX and BAK enables the release of cytochrome c from the mitochondrial intermembrane gap into the cytoplasm, activating caspase (Kerkhofs *et al*., 2021). Cytochrome c binds to apoptotic protease-activating factor 1 (APAF1) by recruitment domain (CARD) and forms apoptosome that activates the initiator caspase 9 (Han *et al*., 2021). The initiator caspase 9 then cleavages (auto cleaves) and becomes active form (Wu *et al*., 2021). The activated initiator caspase 9 then cleaves the inactive effector or executioner caspases (such as caspase 3 and 7) to convert them to active form (tetrameric) (Choi *et al*., 2018).The activated form of caspase 3 cleaves essential proteins in the cell and promotes responsible apoptosis (Kerkhofs *et al*.,2021). All these findings agree with our results, which indicated that the growth or viability of CaCo-2 cells are inhibited due to the intrinsic apoptosis pathway caused by caspase 9 elevation.

Conclusion

The results of our current study showed that the extract of *S. platensis* had concentration dependent antioxidant activity. This extract exerts a concentration dependent anti-proliferation effect on the human colon cancer cell line CaCo-2 and human liver cancer cell line HepG2. The CaCo-2 cells were more sensitive to the extract than the HepG2 cells when compared with normal human fibroblast cell line HdFn, and the extract caused an increase in the activity of caspase 9 in CaCo-2 cell line. These results indicated that the extract of *S. platensis* has antioxidant activity and anti-cancer against colon cancer cell line (CaCo-2) mediated the intrinsic pathway of apoptosis by increasing the caspase 9 in these cells after being treated with extract.

ACKNOWLEDGEMENTS

The authors would like to thank the University of Babylon, College of Science, for providing the required facilities.

Conflict of interest

The authors declare that they have no conflict of interest.

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