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Anticancer Activity of Aqueous and Chloroform Extract of *Spirulina platensis* against SK-GT-4 human cancer cells

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Abstract

Background: Algal derived natural products proved very encouraging in cancer therapy, especially in overwhelming the proliferation and metastasis. Against global health issue that cancer represents, and the side-effects that in so many cases are a feature of established treatments, increasing attention falls on alternative treatments. Deploying bioactive agents from the natural world like these aids efforts to look beyond the traditional drug-based approach.

Methods: The cytotoxicity of *Spirulina platensis* extracts was assessed by MTT assay. Target cells were co-incubated for 72 hours with different extract concentrations. The bioactive components present in these extracts were assessed using GC-MS analysis.

Result: Chloroform extract and aqueous extract obtained from *Spirulina platensis* had cytotoxic activity against SK-GT-4; both concentration and cytotoxicity of the extracts increased. The esophageal cancer SK-GT-4 cell line was more sensitive to extracts than in normal HBL100 cell line. An experiment that made use of gas chromatography-mass spectrometry (GC-MS) discovered that an extract of the plant in its aqueous form had a total of 36 bioactive chemicals, whilst an extract of the plant in its chloroform form included a total of 84 bioactive phytochemical components.

Conclusion: The findings of this study make it abundantly evident that *Spirulina platensis*, despite the fact that it is a natural product, has the potential to be further developed as an anticancer medicine.

Introduction

A correlation was found in several research works between the anticancer properties of algae and their high antioxidant activity. Research revealed that the extracts of the brown algae Dictyota dichotoma displayed considerable levels of cytotoxic activity and anticancer activity [1].

This was the case despite the fact that the extracts had a strong antioxidant power. This conduct is certainly due to hydrophobic anticancer substances, since this seems to be the most likely explanation. It is important to note that some bacteria that live in symbiosis with algae, in addition to medicinal algae, can yield compounds that have the potential to be cytotoxic and anticancer in nature. This is something that should be taken into consideration. One of the kinds of algae known as *Spirulina platensis* is among those that showed a substantial influence on the treatment and inhibition of cancer. Arthrospira platensis, better known to mankind under the name *Spirulina platensis*, represents the group of cyanobacteria—blue-green algae that are made up of multicellular organisms. Cyanobacteria are sometimes referred to by their common name, blue-green algae. Because of its broad nutrient profile consisting of 50–70% protein (on a dry-weight basis), unsaturated fatty acids, liposoluble vitamins, minerals, photosynthetic pigments, storage polysaccharides, β-carotene, astaxanthin, phycocyanobilin, *Spirulina platensis* is widely recognized for its nutritional value [2,3]. The vast majority of these compounds have significant therapeutic potential that might be used for the treatment of inflammatory disorders, cancers, high blood pressure, obesity, high cholesterol, and cardiovascular diseases (CVDs) [4]. It has been shown that certain of these chemicals lower the likelihood of developing cancer [5].

In addition to providing protection from the formation of cancer cells, *Spirulina platensis* also behaves as an agent that provides protection from chemotherapy, as shown by the findings of five separate studies. Several particular medications, whether natural or synthetic, have the capacity to block or even reverse the evolution of carcinogenic pathways prior to the formation of cancer [6]. These drugs have the ability to do this regardless of whether the drug originated in nature or a laboratory. Hosseini et al. (2013) mentioned in their study that the high protein and polyunsaturated fatty acid content of the *Spirulina platensis* algae, in particular the high linolenic acid concentration, imparts several medicinal actions on the product. These therapeutic properties are vital in the treatment of several conditions, like anticancer, antiviral, and antibacterial action, as well as antioxidant, immunomodulatory, and anti-heavy metal activity. It is speculated that the large amount of linolenic acid present in the plant is what is responsible for these pharmacological effects [7].

El-Beltagi et al. (2020) mentioned in their study that *Spirulina platensis* extract has the potential to kill cancer cell lines, and the results showed that it has cytotoxic properties. The CACO, HCT-116, and HepG2 cancer cell lines were investigated. These three distinct cancer cell lines had varied IC50 values, which ranged from 21.8, 14.3, and 11.3. mcg/ml, separately. The presence of antioxidant plant pigments (chlorophyll, phycocyanin, and carotenoids) and polysaccharides was shown to be responsible for this effect. It has been suggested that an extract made from *Spirulina platensis* might be included in the production of anticancer medications. The pigment known as phycocyanin may sometimes be discovered in blue-green algae [8].

Cancer treatments, which are often much more hazardous than the illness itself, may also have unintended side effects. When using natural medicines for the treatment or prevention of illness, reducing the likelihood of experiencing negative responses becomes achievable. According to a study, the influence that *Spirulina platensis* has on the immune system, its ability to repair DNA, and its antioxidant attributes may provide it with anti-cancer properties. According to the amount of work that has already been done, there is an obvious need for more investigation. An alternate method of treating cancer will have its major research emphasis directed towards the production of novel, wholly natural anticancer compounds that are obtained from algae.

The primary focus of the study is whether or not *Spirulina platensis* can slow or stop the cellular growth of cancer.

Methods

Spirulina platensis

Alibaba Company was contacted for *Spirulina platensis* purchase in powder form. For further usage, a refrigerator was employed to store the powdered sample.

Preparation of extracts

In order to extract ethanolic extract of *Spirulina platensis*, the Soxhlet method was used, in which 20g of spirulina powder was placed on the cover and 250 ml ethanol 70% was poured into the balloons of the device. The extraction was performed at 50°C for 3-5 hours, then dried at 40°C. The alcoholic extract was initially mixed with 25 ml of distilled water and similar concentration of chloroform in a separating funnel to achieve phase partitioning into organic and aqueous layers. This process was repeated after the remaining

layer of liquid was added with 30 ml chloroform, with constant agitation on a magnetic stirrer for 48 hours. This final mixture was returned to a separating funnel, and the chloroform (organic) phase was separated and concentrated to obtain the chloroform extract. The remaining aqueous phase was further treated with equal volume of ethyl acetate and stirred for 48 more hours. This mixture was subjected to separation with the aid of a funnel, and clear aqueous and ethyl acetate layers were recovered. The aqueous layer was finally dried to obtain the aqueous extract.

Gas Chromatography-Mass spectrometry analysis (GC-MS)

The aqueous and chloroform extracts of *Spirulina platensis* were analyzed for chemical composition with gas chromatography (GC) in an Agilent 7890 B system that was integrated with an Agilent 5977 A mass selective detector (MSD). This analytical instrument, operated using Mass Hunter GC/MS Acquisition software (USA), was established at Basra Oil Company's Nahran Omar Laboratories. The apparatus was equipped with a 5 DB-MS capillary column of fused silica, with a length of 30 meters, film thickness of 0.25 µm, and 0.32 mm internal diameter. Carrier gas used was helium with 99.9% purity. Compound resolution was in compliance with the following GC-MS thermal gradient: oven temperature was maintained constant at 40°C for 5 minutes, following which it was ramped up to 150°C and subsequently to 250°C at a rate of 4°C per minute. Compounds so identified from chromatograms were calculated in proportion to their respective relative peak areas and expressed in percentages [9].

Cytotoxic test

SK-GT-4 human esophageal carcinoma cells and HBL-100 normal human breast epithelial cells were provided by the College of Education for Pure Sciences, Basra University, tissue culture lab. In order to screen for cell proliferation and viability, we used the in vitro, reliable, and sensitive MTT assay. This in vitro assay is routinely employed to identify alterations in cell health, like inhibition of cell proliferation, apoptotic cell death, or necrotic cell death, based on the assessment of the metabolic activity of cells [10]. MTT is used in these assays. In an incubator set to 37 degrees Celsius with 5% carbon dioxide, 96-well plates were seeded with 1.0 x 104 cells per well, and the culture was allowed to continue for 24 hours. The cells were treated with extracts made from chloroform and aqueous medium at a number of different concentrations, including 15.5, 31, 62.5, and 125 µg/ml. Each concentration of extracts was analyzed in four duplicate sets in order to make the results exact and reproducible. Treated cells remained in standardized conditions of 37°C and 5% CO_2 for 27 hours. After the stipulated time had elapsed, the supernatant was removed, and 100 µl of MTT solution was added. The mixture was then placed in an incubator set to 37°C for a period of 4 hours. After that, 100 liters worth of dimethyl sulfoxide (also known as DMSO) was added to the mixture, and stirred for ten minutes at room temperature. At a wavelength of 620 nm, an ELISA reader was used in order to get the absorbance reading. The following equation was utilized to assess the percentage of viable cells at each concentration:

viability % = the absorbance of treated / the absorbance of control × 100.

The half-maximal inhibitory concentration (IC_{50}) for each extract was determined through GraphPad Prism software [11].

Statistical analysis

Mean and standard error of means are used to describe the findings. To find differences between the treated and control cell lines, one-way ANOVA was used. The values of $P < 0.05$ were considered statistically significant for the analysed data.

Results

GC-MS Analysis

GC-MS analysis conducted for both chloroform and aqueous extracts of *Spirulina platensis* indicated that the chloroform extract contained 84 bioactive compounds, while the aqueous extract contained 36 compounds, as shown in Figures 1&2. The major constituents in the chloroform extract were hexadecanoic acid, ethyl ester (14.77%), neophytadiene (9.90%), oleic acid (6.87%), (E)-9-octadecenoic acid, ethyl ester (4.56%), and phytol (3.10%). Detailed identification and area percentage of each compound are listed in Table 1, and representative chemical structures are shown in Table 2. In contrast, the aqueous extract had significant amounts of 9-octadecenoic acid, methyl ester (23.17%), hexadecanoic acid, methyl ester (18.18%), dodecanoic acid, methyl ester (11.68%), and methyl tetradecanoate (8.22%).

Cytotoxicity Assay and Cell Viability

The in vitro cytotoxicity of *Spirulina platensis* extracts was assayed comparatively against the SK-GT-4 and the HBL-100 cells.

Peak	Retention time	Area %	Compound name
1	5.075	0.154	Acetic acid
2	5.179	0.7359	Trichloronitromethane
3	5.378	0.2323	Cyclopentane, 1,2-dimethyl-, trans-
4	5.474	0.1608	Decyl octyl ether
5	6.904	0.1734	1-Octene
6	7.089	0.4906	Hexanal
7	8.896	0.1708	Cyclopropane, 1-methyl-2-pentyl-
8	10.26	0.151	Benzene, 1-ethyl-2-methyl-
9	10.799	0.2095	Furan, 2-pentyl-
10	10.85	0.178	Mesitylene
11	10.939	0.3009	Decane
12	12.687	0.4613	Undecane
13	13.675	0.2904	Isopinocarveol
14	14.125	0.2846	Ethanone, 1-(4-methylphenyl)-
15	14.272	0.1571	Dodecane
16	14.84	0.1711	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-
17	15.733	0.2198	Tridecane
18	16.78	0.2282	n-Decanoic acid
19	18.27	0.5216	Dodecanenitrile
20	18.366	0.5981	Pentadecane
21	18.579	0.6071	2,4-Di-tert-butylphenol
22	18.668	0.4647	Dodecanoic acid, methyl ester
23	18.978	0.8898	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-
24	19.236	0.8136	Dodecanoic acid
25	19.516	1.4431	Dodecanoic acid
26	19.745	0.225	Tetradecanal
27	20.394	0.2128	1,13-Tetradecadiene
28	20.475	0.908	8-Heptadecene
29	20.534	0.3728	8-Heptadecene
30	20.645	0.6222	hexadecyl acrylate
31	20.711	0.8287	Heptadecane
32	20.984	0.3833	Methyl tetradecanoate
33	21.412	0.4983	Tetradecanoic acid
34	21.729	2.3274	Tetradecanoic acid, ethyl ester
35	22.245	9.9027	Neophytadiene
36	22.311	2.1553	2-Pentadecanone, 6,10,14-trimethyl-
37	22.474	1.1846	1-Hexadecyne
38	22.584	0.1483	Dibutyl phthalate
39	22.673	4.6194	9-Octadecyne
40	22.739	0.2675	d-Gulopyranoside, 2,3:4,6-di-O-(ethylboranediyl)-1-O-methyl-
41	22.865	0.9672	Pentadecanenitrile
42	23.086	1.6758	Hexadecanoic acid, methyl ester
43	23.314	0.1521	13-Tetradecen-1-ol acetate
44	23.565	3.7381	n-Hexadecanoic acid
45	23.801	14.7717	Hexadecanoic acid, ethyl ester
46	24.524	0.4014	Z-8-Methyl-9-tetradecenoic acid
47	24.59	0.3935	E,Z-1,3,12-Nonadecatriene
48	24.627	0.6939	9-Octadecenenitrile, (Z)-
49	24.782	1.7921	9-Octadecenoic acid, methyl ester, (E)-
50	24.834	0.9172	9-Octadecenoic acid (Z)-, methyl ester
51	24.915	3.1094	Phytol
52	24.996	0.4878	Heptadecanoic acid, 14-methyl-, methyl ester
53	25.188	2.5324	Oleic Acid
54	25.35	6.8785	Oleic Acid
55	25.468	4.5658	(E)-9-Octadecenoic acid ethyl ester
56	25.616	4.6288	Octadecanoic acid, ethyl ester
57	25.837	0.4541	Neophytadiene
58	26.353	0.2475	15-Hydroxypentadecanoic acid
59	26.501	0.2808	9-Octadecenoic acid, (E)-
60	26.987	0.2408	Oleic Acid
61	27.105	1.1781	9-Octadecenamide, (Z)-
62	27.275	1.4895	Cyclotetracosane
63	27.961	0.2999	Oleic Acid
64	28.035	0.8686	Myristoyl chloride
65	28.234	0.5565	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
66	28.551	0.761	Bis(2-ethylhexyl) phthalate
67	28.831	1.0262	1-Hexacosene
68	29.001	0.1617	Eicosanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[(1-oxohexadecyl)oxy]methyl]ethyl ester
69	29.421	0.3916	Oleic Acid
70	29.48	0.3383	2,4-thiophenedicarboxaldehyde, 5-(dimethylamino)-
71	29.591	0.2626	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester
72	30.277	0.6564	1-Hexacosene
73	30.896	0.5153	16-Hentriacontanone
74	31.479	0.3049	Hexadecanoic acid, tetradecyl ester
75	31.671	0.1984	1-Nonadecene
76	32.231	0.7577	Succinic acid, dodec-2-en-1-yl 2,2,3,4,4,4-hexafluorobutyl ester
77	32.393	0.3328	Nonacosan-14-one
78	33.087	0.6334	Phytyl dodecanoate
79	34.141	0.2412	Cyclopentane, 1,1'-hexadecylidenebis-
80	34.363	0.4287	16-Hentriacontanone
81	35.255	0.1752	Phytyl heptadecanoate
82	36.811	0.7054	Hentriacont-7-en-16-one
83	38.426	3.1069	Hexadecanoic acid, 3,7,11,15-tetramethyl-2-hexadecenyl ester
84	39.636	0.9169	Tris(2,4-di-tert-butylphenyl) phosphate

Table 1: Bioactive compounds detected in chloroform extract of Spirulina platensis by GC-MS analysis.

Peak	Retention time	Area %	Compound name
1	7.524	1.3671	Dimethyl ether
2	7.642	4.7425	Methanamine, N-hydroxy-N-methyl-
3	7.833	1.5432	Silane, dimethoxymethyl-
4	8.099	0.2801	Propylene Glycol
5	8.151	0.2861	1-Cyclopentylacetonitrile
6	8.232	0.2613	Butanoic acid, 2-hydroxy-, ethyl ester
7	8.637	1.2234	2-Cyclopenten-1-one
8	9.25	0.5049	2-Cyclopenten-1-one, 2-methyl-
9	10.924	0.2398	Cyclotetrasiloxane, octamethyl-

10	13.107	0.3983	Isophorone
11	13.564	0.3398	Cyclopentasiloxane, decamethyl-
12	14.198	0.6142	2-n-Butyl-2-cyclopentenone
13	16.086	0.9722	Cyclohexasiloxane, dodecamethyl-
14	16.19	0.3897	1-Azabicyclo[2.2.2]octane, 3-methyl-
15	16.389	0.4965	N-n-Propylmaleimide
16	18.336	0.6283	Cycloheptasiloxane, tetradecamethyl-
17	18.572	0.5192	2,4-Di-tert-butylphenol
18	18.675	11.6815	Dodecanoic acid, methyl ester
19	19.745	0.2729	1(2H)-Naphthalenone, 3,4-dihydro-3,3,6,8-tetramethyl-
20	20.984	8.2208	Methyl tetradecanoate
21	22.267	1.0992	2-Pentadecanone, 6,10,14-trimethyl-
22	22.319	0.2884	1-Hexacosene
23	22.614	1.7826	Cyclohexadecane
24	22.842	0.3058	Octadecanenitrile
25	23.086	18.1874	Hexadecanoic acid, methyl ester
26	24.59	2.9236	Behenic alcohol
27	24.708	0.5288	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
28	24.775	23.1785	9-Octadecenoic acid (Z)-, methyl ester
29	24.819	7.7139	cis-13-Octadecenoic acid, methyl ester
30	24.885	2.9599	Phytol
31	24.974	3.8953	Methyl stearate
32	25.106	0.4471	Heptanoic acid, 7-(o-pentylphenyl)-, methyl ester
33	25.542	0.5437	9,11-Octadecadienoic acid, methyl ester, (E,E)-
34	26.714	0.3866	Eicosanoic acid, methyl ester
35	27.054	0.2808	9-Octadecenamide, (Z)-

Table 2: Bioactive compounds detected in aqueous extract of Spirulina platensis by GC-MS analysis.

Concentration µg/ml	Chloroform extract		Aqueous extract	
	Mean ±SD		Mean ±SD	
	SK-GT-4	HBL100	SK-GT-4	HBL100
Control	98.9 ± 2.1^{A}	98.2 ± 3.2^{A}	98.9 ± 2.1^{A}	98.2 ± 3.2^{A}
15.5	91.22 ± 13.63^{A}	96.62 ± 8.85^{A}	96.63 ± 10.69^{A}	97.85 ± 5.25^{A}
31.0	80.24 ± 2.07^{B}	93.75 ± 4.89^{AB}	96.39 ± 8.19^{A}	96.36 ± 14.4^{A}
62.5	60.02 ± 5.00^{C}	83.21 ± 17.8^{BC}	95.34 ± 2.65^{A}	83.70 ± 13.23^{B}
125.0	36.43 ± 1.84^{D}	75.77 ± 10.19^{C}	48.48 ± 7.90^{B}	70.10 ± 5.39^{C}
P value	0.001 † HS	0.072 † NS	0.001 † HS	0.007 † NS

Table 3: Cell lines' viability percentage after treatment with chloroform and aqueous extracts of Spirulina platensis.
Different letters denote the significant differences at P < 0.05.
SD: standard deviation; †: one way ANOVA; HS: Highly significant at P < 0.001; NS: non-significant at P > 0.05

Chloroform Extract

The cell viability of SK-GT-4 significantly decreased with growing quantity of extract (from 91.22% at 15.5 µg/ml to 36.43% at 125 µg/ml) as presented in Table 3. In contrast, normal HBL100 cells showed less reduction in viability (from 96.62% to 75.77%) over the same concentration range, as shown in Table 3. The IC_{50} value for SK-GT-4 cells treated with chloroform extract was 53.83 µg/ml.

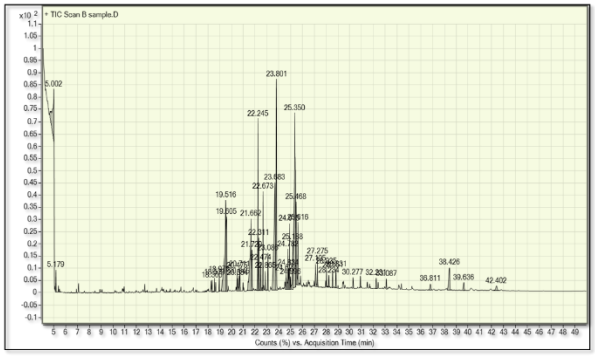


Figure 1: GC-MS analysis in the Chloroform extract of *Spirulina platensis*.

Aqueous Extract

In the SK-GT-4 cancer cells, viability remained largely unchanged at concentrations up to 62.5 µg/ml but dropped to 48.48% at 125 µg/ml (Table 3). The HBL100 cell line demonstrated minimal effects at lower doses, with a decrease to 70.10% viability only at the highest concentration (125 µg/ml), as shown in Table 3. The IC_{50} for the aqueous extract was 72.84 µg/ml.

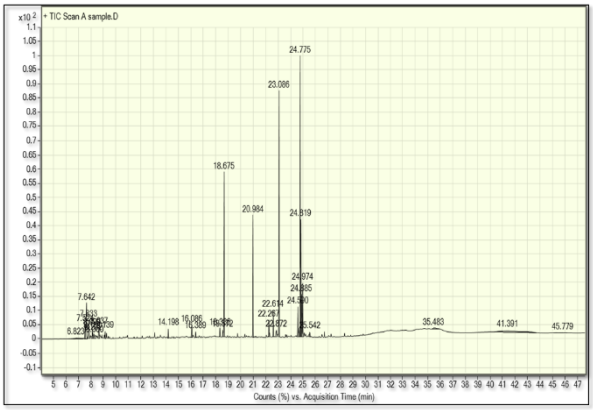


Figure 2: GC-MS analysis in the aqueous extract of *Spirulina platensis*.

Discussion

The occurrence of several bioactive compounds was established by the GC-MS analysis in both chloroform and aqueous extracts of *Spirulina platensis*, which were compared against the NIST library and standard references for compound identification [12]. The chloroform extract showed a broader chemical diversity

(84 compounds) than the aqueous extract (35 compounds), as detailed in Figures 1 and 2 and Tables 1 & 2. Major constituents in the chloroform extract included hexadecanoic acid, ethyl ester (14.77%), neophytadiene (9.90%), oleic acid (6.87%), (E)-9-octadecenoic acid, ethyl ester (4.56%), and phytol (3.10%). The aqueous extract contained dominant compounds such as 9-octadecenoic acid methyl ester (23.17%) and hexadecanoic acid methyl ester (18.18%). These results align with the findings of El Din SM (2019) [13].

Cytotoxicity results indicated a concentration-dependent reduction in viability of SK-GT-4 cancer cells treated with the chloroform extract, with significant effects observed at concentrations ⩾ 31.0 µg/ml (Table 3). In contrast, HBL100 normal cells showed minimal cytotoxicity under the same treatment (Table 3). The calculated IC_{50} for SK-GT-4 was 53.83 µg/ml. These outcomes are consistent with those reported by MZ MS (2012), who demonstrated selective cytotoxicity of chloroform microalgal extracts on cancer cell lines [14]. The observed anticancer effects may be attributed to compounds such as 9-octadecenoic acid, oleic acid, ethyl ester of hexadecanoic acid, and n-hexadecanoic acid [15-23]. The anticancer potential of fatty acids, including hexadecanoic acid, has been reported in various seaweed species and is associated with induction of apoptosis through activation of caspase-3, caspase-8, and Bax proteins [24,25].

Furthermore, studies demonstrated the cytotoxic effect of Scenedesmus sp. and S. obliquus extracts against HepG2, MCF7, and CT116 cancer cell lines [26,27]. The presence of fatty acid esters enhances the biological activity of algal extracts [28,29]. Aqueous extract treatment also influenced cell viability, albeit to a lesser extent. Only the highest concentration (125 µg/ml) significantly reduced viability in SK-GT-4 cells (Table 3), with an IC_{50} value of 72.84 µg/ml. HBL100 cells showed a more marked viability reduction at concentrations ⩾ 62.5 µg/ml (Table 3). These results agree with Abo El-Sayed B (2022), who observed modest anticancer activity in aqueous extracts [30], and with MZ MS (2012), who validated the anticancer potential of microalgae [14]. Bioactive constituents in the aqueous extract include methenamine, N-hydroxy-N-methyl, methyl tetradecanoate, phytol, behenic alcohol, and 9-octadecenoic acid (Z)-methyl ester [31]. These compounds likely contribute to the extract's cytotoxicity. As indicated by the spectrum of alcohols, phenols, esters, and fatty acids identified, these components may be responsible for modulating anticancer effects [32]. This suggests that crude extracts of *Spirulina platensis* could serve as valuable resources for anticancer drug development.

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Author Contributions

Mohammed A. Auda conceptualized the review, developed the framework, and provided oversight for the manuscript. Rasha N. Aljabery conducted the primary literature search, data analysis, synthesis, and background sections, while Hussain Yousif Al-Rekabi focused on the discussion and future perspectives. The revision, correction, and final version approval of the manuscript were conducted by all authors equally.

Competing Interest

The authors declare no conflict of interest in the publication of this manuscript.

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