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Bioprospecting of microalgae as anticancer, antioxidant, and antidiabetic agents

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Abstract

Algae, either macroscopic or microscopic, often grow rapidly across diverse ecosystems. These organisms offer promising potential in the pharmaceutical and nutraceutical fields due to their valuable bioactive compounds such as astaxanthin, phycocyanin, stigmasterol, polysaccharides, steroids, and minerals. The bioactive compounds play a vital role in scavenging free radicals by binding to their molecules and targeting damaged cells. Additionally, some reports have highlighted the substantial anticancer activity of microalgae. Cancer, characterized by abnormal cell growth in tissues, bones, and blood, can be targeted through certain microalgal bioactive compounds that interact with p53 and Bcl2 proteins to induce apoptosis in the affected cells. Furthermore, microalgal secondary metabolites exhibit antidiabetic effects by stimulating insulin secretion in pancreatic beta cells, synthesizing insulin-like proteins, and mitigating insulin resistance. These microorganisms are potential sources for producing novel green therapeutic agents to combat degenerative diseases. Therefore, optimizing the application of microalgal biorefinery within the pharmaceutical industry is crucial.

Keywords: Bioactive compounds, Cancerous cells, Insulins, Proteins

1. Introduction

Microalgae, a subset of microorganisms prevalent in marine environments, are commonly categorized as plankton. They inhabit various aquatic ecosystems, including marine, freshwater, brackish water, and some surfaces such as stones. Microalgae possess numerous essential components often applied in medicine, for instance, *Chlorella* contains lutein, carotene, zeaxanthin, and astaxanthin that form carotenoids with anticancer and antioxidant properties [1].

Some microalgae have garnered attention for their potential in treating cancer and diabetes. Microalgae extracts are primarily used to induce apoptosis, the programmed death of cancer cells characterized by cell shrinkage, deformation, and chromatin condensation. Ethanol and ethyl acetate extracts of *Chaetoceros calcitrans* exhibit effects similar to tamoxifen, a conventional therapeutic agent for breast cancer treatment [2]. Fatty alcohol ester nonyl 8-acetoxy-6-methyl octanoate (NAMO) derived from *P. tricornutum* demonstrated anticancer effects across three different cancer cell lines [3]. Ethanol extract of *Chloromonas* sp. has been evaluated on cervical, melanoma, and breast cancer cells [4].

The extract of Canadian marine microalgae exhibited anticolonial activity and enhanced apoptosis when tested in vitro. Additionally, in vivo studies reported antimetastatic effects in NOD-SCID mice [5]. Caspase-3, a crucial member of the caspase family of cysteine proteases, plays a pivotal role in the apoptotic mechanism [4]. Apoptosis signaling pathways are known to involve measuring caspase-3, Bcl-2, and P53 proteins. The concentration of extracts has been discovered to influence their effects. For example, *Chloromonas* ethanol extract exerted significant cytotoxic effects on melanoma and cervical cancer cells, with anti-proliferative effects observed at a higher concentration 12,5 [4].

Beyond the anticancer potential, microalgae also exhibit antidiabetic activity. Microalgal bioactive agents inhibit carbohydrate hydrolyzing enzymes, such as amylase and α-glucosidase [6]. *Spirulina platensis* extract increases trace minerals (TM) and antioxidant enzymes, reducing glucose concentration, lipids, and inflammatory

responses, where lipid peroxidation and hyperglycemia are diabetes indicators [7]. Therefore, this review aims to comprehensively explore the antidiabetic, anticancer, and antioxidant medical properties of microalgae to promote the maximization of their benefits.

2. Bioprospecting of microalgae

Microalgae, commonly inhabiting both freshwater and marine environments, contain many valuable nutritional compounds. These microorganisms are currently used extensively across several industrial activities, specifically in the pharmaceutical and nutraceutical fields. Their demand increases consistently due to being a source of bioactive ingredients applied as anti-inflammatory, antioxidant, antimicrobial, and anticancer agents (Figure 1) [8,9]. Important pigments vital to the pharmaceutical sector, including astaxanthin, phycocyanin, and stigmasterol, are derived from microalgae [8].

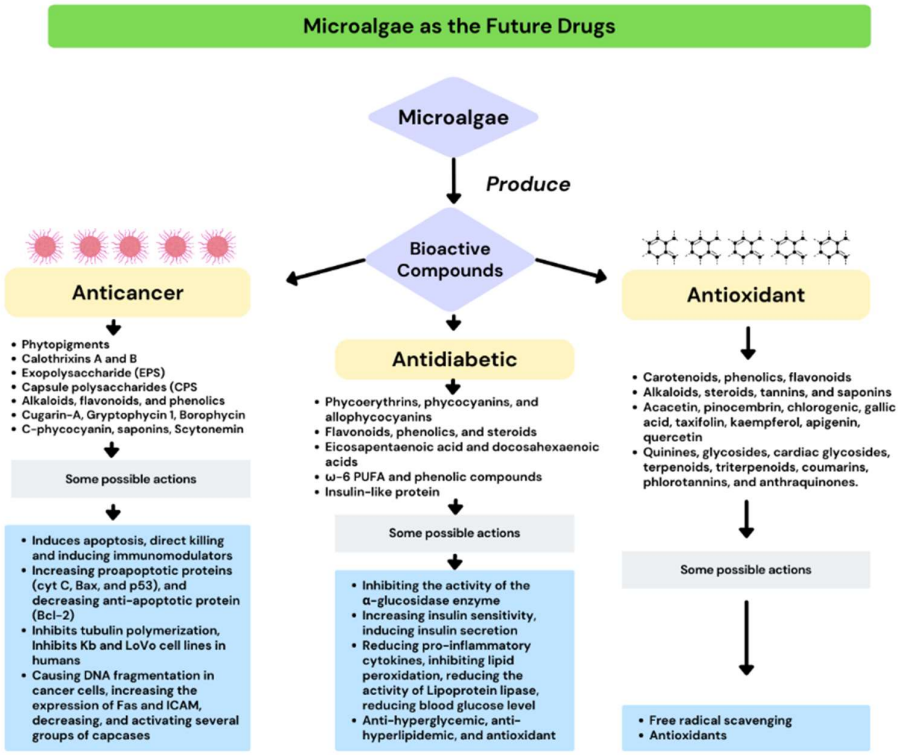

Figure 1 Bioprospecting of miocroalgae as anticancer, antidiabetic, and antioxidant.

Microalgae are often classified as plankton, certain diminutive organisms that float in water bodies. Freshwater microalgae are up to 10 major phyla, while those found in seawater are over 30,000 different species each bearing untapped potential [10]. Microalgae demonstrate numerous applications due to their primary and secondary metabolites commonly employed as feedstock in various biorefinery activities. The rapid growth rate of these microorganisms prompted their mass or industrial-scale cultivation using wastewater containing various organic compounds. Several microalgal strains have been reported to exhibit high bioprospecting potential, particularly within the agriculture, pharmaceuticals, health, and energy sectors. However, the enhancement of strain capability and productivity remains paramount. Meticulous attention to the physical conditions of the growth environment is essential for optimizing growth rates and biomass yield [11].

3. Bioprospecting of microalgae as anticancer agents

Errors in the cell cycle are one of the factors initiating cancer, which is conventionally treated by radiation and chemotherapy, both bearing negative impacts on patients. Consequently, there is a need for novel natural anticancer agents such as microalgae, a source of bioactive compounds, including polysaccharides, steroids, vitamins, minerals, and more. Several previous studies have documented the anticancer activity of microalgae extracts, often exhibited through cell cycle inhibition [10], as presented in Table 1.

Table 1 Some microalgae used as anticancer agents.

No.	Species	Bioactive compounds	Cell Lines	Actions	Reference
1.	*Arthrospira platensis*	Phytopigments	HeLa	Induces apoptosis	[12]
2.	*Calothrix* sp	Calothrixins A and B	HeLa	Controlling the cell cycle and mitotic division	[10,13]
3.	*Chlorella pyrenoidosa*	Exopolysaccharide (EPS)	Colon cancer cells HCT116 and HCT8	Antioxidants and antitumor	[14]
4.	*Chlorococcum* sp.	exopolysaccharide (EPS)	colon cancer cells HCT116 and HCT8	Antioxidants and antitumor	[14]
5.	Cyanobacteria	Capsule polysaccharides (CPS) and extracellular polysaccharides (EPS)	Human cancer cell lines	Anti-inflammatory and antioxidant agents	[15]
6.	*Euglena tuba*	Alkaloids, flavonoids, and phenolics.	Dalton's Lymphoma	Increasing proapoptotic proteins such as cyt C, Bax, and p53, and decreasing anti-apoptotic protein (Bcl-2)	[16]
7.	*Lygbya martensiana*	1-monolinoleoyilglycerol trimethylsilyl ether and n-Hexadecanoic acid	HepG2	Antioxidant and antibacteria	[17]
8.	*Lyngbia* sp.	Cugarin-A	Colon, breast, and kidney cancer cells	Inhibits tubulin polymerization	[10,18]
9.	*Lyngbya majuscule*	1-monolinoleoyilglycerol trimethylsilyl ether and n-Hexadecanoic acid	HepG2	Antioxidant and antibacteria	[17]
10.	*Nostoc Linckia*	Borophycin	Kb and LoVo cell lines	Inhibits Kb and LoVo cell lines in humans	[10,19,20]
11.	*Nostoc* sp.	Gryptophycin 1	Human tumor cell lines	Antiproliferative agent	[10,21,22]
12.	*Nostoc spongiaeformae* var. *tenue*	Borophycin	Kb and LoVo cell lines	Inhibits Kb and LoVo cell lines in humans	[10,19,20]
13.	*Parachlorella kessleri*	Extracellular polysaccharides	CT26 colon carcinoma	Direct killing and inducing immunomodulators	[23]
14.	*Scenedesmus* sp.	exopolysaccharide (EPS)	HCT116 and HCT8	Antioxidants and antitumor	[14]
15.	*Spirulina platensis*	C-phycocyanin	HeLa and MCF7	Causing DNA fragmentation in cancer cells, increasing the expression of Fas and ICAM, decreasing BCL-2 expression, and activating several groups of caspases	[10]
16.	*Spirulina* sp.	Phycocyanins, polysaccharides, flavonoids, resins, saponins, and alkaloids,	Breast cancer cells	Enhancing the work of the immune system, repairing damaged DNA, acting as antioxidants, and triggering apoptosis in cancer cells	[24]
17.	*Stigonema* sp.	Scytonemin	HeLa	Antiproliferative and anti-inflammatory	[10,13]

Anticancer remedies can generally be in the form of antitumor agents derived from blue-green algae, carotenoids, polyunsaturated fatty acids (PUFAs), polysaccharides, and peptides [10]. Among Cyanophyceae, *Nostoc* sp. produces Gryptophycin 1 which inhibits human tumor cell lines by acting as an antiproliferative agent [10,21,22]. Additionally, borophycin extracted from *Nostoc spongiaeformae* var. *tenue* and *N. Linckia,* curbs human Kb and LoVo cell lines [10,19,20]. Cugarin-A, isolated from *Lyngbia*, hinders tubulin polymerization, effectively suppressing colon, breast, and kidney cancer cell growth [10,18]. Compounds such as Calothrixins A and B from *Calothrix* sp., and Scytonemin from *Stigonema* sp., control cell cycle and mitotic division [10,13], yielding antiproliferative and anti-inflammatory effects. C-phycocyanin obtained from *Spirulina platensis* causes DNA fragmentation in cancer cells, increases the expression of Fas and ICAM, decreases BCL-2 expression, and activates several groups of caspases, effectively inhibiting HeLa and MCF7 cells [10].

Microalgae-derived carotenoids such as astaxanthin prove effective in colorectal cancer treatment by stimulating apoptosis in cancer cells. The apoptosis is induced through pathways involving Nuclear factor Kappa B (NF-Kb), matrix metalloproteinase 9 (MMP9), cyclooxygenase 2 (COX-2), matrix metalloproteinase 2 (MMP2), Extracellular signal-regulated kinase (ERK-2), and AKT activity [10,25]. Additionally, astaxanthin effectively prevents cancer cells from remaining within the G0/G1 growth phase. Fucoxanthin, another carotenoid, inhibits leukemic-HL 60 cells [10,26] and increases cancer cell death by activating caspase-3, mitigating Bax and Bcl-2 activity, and prompting DNA fragmentation [10].

Arthrospira, a globally utilized Cyanobacteria, has been reported to exhibit anticancer activity. Oral administration of this strain at 250-550 mg/kg^{-1} body weight prevents conformational changes in hepatocellular carcinoma (HCC), reduces tumor AAFP biomarkers, decreases the size and number of nodules, and enhances survival rates in HCC-induced rats, as well as elevates antioxidant activity. Similarly, *Spirulina* provides positive effects on diverse cancer cell types including HepG-2, MCF-7, SGC-79-01, A549, and HT-29 [27,28]. *Spirulina* extract restricts breast and lung cancer growth, inhibits HCC through mitochondria-mediated apoptosis, and induces apoptosis in oral cancer by curbing cell angiogenesis and proliferation. The mechanisms involve modulation of nuclear factor kappa-light-chain-enhancer of activated B-cells (Nf-kB), Extracellular signal-regulated kinases (ERK), and Janus Kinase / Signal transducers and activators of transcription (JAK-2/STAT-3)[27].

Spirulina sp. also produces phycocyanins, polysaccharides, flavonoids, resins, saponins, and alkaloids that boost immune function, repair damaged DNA, act as antioxidants, and trigger cancer cell apoptosis [24]. Bioactive compounds synthesized by Cyanobacteria contribute to intra- and extracellular anti-inflammatory mechanisms, by counteracting free radicals, modulating cyclooxygenase-2 expression, and downregulating pro-inflammatory cytokines. Polysaccharides from this organism inhibit pro-inflammatory cytokine activity and exist as capsule polysaccharides (CPS) on cell surfaces or extracellular polysaccharides (EPS) excreted into the cellular environment [15].

Polysaccharides such as D-galactan sulfate from *Gymnodinium* sp., demonstrate anticancer activity by inhibiting DNA topoisomerase I and II in cancer cells [10,29]. Peptides derived from microalgae enhance antioxidant activity [10], while produced lipids exhibit anticancer properties. For instance, synthesized docosahexaenoic acid (DHA) boosts lipid peroxidase activity, inducing stress on the nucleus and mitochondria of cancer cells. This triggers changes in mitochondrial structure and function, leading to apoptosis [10]. Lipid peroxidation alters the membrane potential and structure of these abnormal cells. Furthermore, PUFAs influence apoptosis rate through ERK activation, reducing intracellular levels of cytochrome c, p53, and Bax, which halt cell cycle and proliferation [10,31,32].

Marine microalgae isolates, *Lyngbya majuscule* and *Lygbya martensiana,* display antioxidant activity with IC_{50} values of 251.34 and 282.24 $\mu g/mL^{-1}$, respectively, while *Lygbya majuscule* further exhibits a reducing power of 0.76. These strains yield secondary metabolites comprising phenolics and flavonoids. Within their extract, bioactive compounds such as 1-monolinoleoyilglycerol trimethylsilyl ether and n-Hexadecanoic acid were found to manifest efficacy against tested microbes and HepG2 cancer cells, with a cytotoxic value of 88.2% at a 15.62 $\mu g/mL^{-1}$ concentration. These compounds spare normal cells, while the methanol extract serves as an antibacterial agent, with a minimum inhibitory concentration (MIC) against *Vibrio cholerae*, *E. Coli,* and *Candida albicans* ranging from 62.5 $\mu g/mL^{-1}$ to 500 $\mu g/mL^{-1}$. The aforementioned secondary metabolites demonstrate efficacy against Gram-positive and negative bacteria [17].

Some strains including *Chlorella sorokiniana*, *Dunaliela* sp., and various Cyanobacteria, such as *Dolichospermum flos-aquae, Dolichospermum crassum, Dolichospermum spiroides, Dolichospermum circinale, Oscillatoria* sp*., Oscillatoria nigro-viridis, Oscillatoria sancta, Anabaena oryzae, Anabaena* sp., *Leptolygbya fragilis, Aphanizomenon gracile*, and *Wollea saccata* also produce phenolic compounds. *Dolicospermum circinale* displayed strong antioxidant activity with an IC_{50} of 17.2 $\mu g/mL^{-1}$, while *Dolichospermum flos-aquae* presented the highest NO scavenging activity with an IC_{50} of 28.7 $\mu g/mL^{-1}$. A substantial lipid peroxidase effect was found in Dolichospermum flos-aquae ($IC_{50} = 11.9$ $\mu g/mL^{-1}$) and the most significant cytotoxicity against peripheral blood mononuclear cells (PBMCs) was detected in the *Leptolyngbia fragilis* isolate ($IC_{50} = 27.8$ $\mu g/mL^{-1}$). *Dolichospermum crassum* showed the greatest anticancer activity against colon (Caco-2), breast (MCF-

7), liver (HepG-2), and prostate (PC3) cancer cells, with IC_{50} values of 57.9, 41.1, 68.6, and 44.1 µg mL^{-1}, respectively. This activity surpassed the type exhibited by the positive control known as 5-fluorouracil [31]

The aqueous extract of *Chlorella vulgaris* reduced HeLa cancer cell viability by 68% at 1000 µg/mL. Meanwhile, *Arthrospira platensis* exhibited 39% cytotoxicity against the same cell line at a similar concentration level. Phytopigment content facilitates the antioxidant activity of microalgae extracts, with concentration playing a role in determining the resulting effectiveness [12].

Chlorella pyrenoidosa, *Chlorococcum* sp., and *Scenedesmus* sp. produce exopolysaccharides (EPS) containing several monosaccharides, such as mannose, ribose, rhamnose, glucose, glucosamine, and galactose. The EPS compound absorbs heavy metals from the environment, functions as antioxidants for free radical scavenging, and exhibits antitumor properties by reducing the viability of human colon cancer cells HCT116 and HCT8. The administration of EPS at a 0.6 mg/mL^{-1} concentration decreased the viability of HCT116 by 10% and HCT8 by 20-35%. Among these species, EPS from *Scenedesmus* sp. at a concentration of 1.5 mg/mL^{-1} demonstrated the highest efficacy in curtailing HCT116 HCT8 and HCT116 colony formation by 42.8% and 35.2%, respectively. These observations indicated the antitumor potential of EPS derived from the three microalgae species [14].

The aqueous extracts of *Desmococcus, Chlorella,* and *Scenedesmus* have relatively high antimicrobial and anticancer activity. Furthermore, *D. olivaceus* restricted *Staphylococcus aureus* and *Pseudomonas syringiae* growth, with inhibition zones reaching 6.7 and 5.3 mm, respectively, while promoting *Bacillus thrungiensis* growth with stimulation zones of 16.7 mm. The extracts effectively inhibited human cancer cells, including MCF7, CEM, G361, and NIH3T3. Specifically, LC50 of *Scenedesmus* sp. against MCF7 and CEM was 0.07 and 0.06 mg $well^{-1}$, respectively. The most effective LC50 for G361 cell lines was 0.17 mg $well^{-1}$ from *Scenedesmus* sp, while the lowest value for NIH3T3 was 1.15 mg $well^{-1}$ from *Chlorella* sp. [11].

Euglena tuba produces bioactive compounds, including 1-Hexadecanol, methyl methyltetradecanoate, Phytalic acid, hexadecanoic acid, and methyl ester. These compounds elevate the expression of the pro-apoptotic protein Bax, induce cytochrome C production within mitochondria, reduce membrane potential, and trigger intrinsic apoptosis. Additionally, they instigate changes in cancer cell morphology, stimulating apoptosis through nuclear fragmentation, nucleus damage, and chromatin condensation. The bioactive compounds decrease the expression of the anti-apoptotic protein Bcl2 while increasing P53 expression, which promotes apoptosis [16].

Previously conducted in vivo studies regarding microalgae anticancer potential [23] examined the ability of extracellular polysaccharides from *Parachlorella kessleri* to inhibit the growth of CT26 colon carcinoma cells in mice. Intraperitoneal injection of these polysaccharides at 10 mg kg^{-1} twice a week weakens the cancer cells, impeding their growth through direct elimination. Additionally, polysaccharides stimulate the immune system to fight against cancer cells.

Some reports suggest that microalgae possess substantial potential to be developed as anticancer agents due to their array of bioactive compounds targeting various pathways or mechanisms to inhibit or eliminate cancer cells. These mechanisms encompass apoptosis induction, angiogenesis prevention, metastasis suppression, and direct cytotoxicity. However, further investigation is required on in vivo efficacy and direct patient application. There is a need to assess the safety and side effects of employing microalgae as anticancer agents.

4. Bioprospecting of microalgae as antidiabetic agents

Diabetes, a disease caused by a lack of insulin secretion or hyperglycemia, can be controlled using Microalgae, as presented in Table 2. Administration of *Nannochloropsis gaditana* was reported to increase food and energy intake in diabetic-induced rats compared to their normal counterparts. A 10% supplement of microalgae successfully augmented body weight in diabetes-induced rats and mitigated diabetic symptoms. Observations show that enhanced energy and food intake in rats translates to efficient energy absorption. *N. gaditama* supplementation reduced several diabetes parameters such as glucose, cholesterol, LDH, urea, creatinine, and uric acid levels, and restricted pro-inflammatory cytokine activity. Conversely, this supplement boosted antioxidant enzyme activity and decreased malondialdehyde (MDA) and carbonyl protein levels within the rat livers [32].

The proficiency of microalgae in reducing blood glucose levels can be attributed to their ability to induce insulin hormone secretion in pancreatic beta cells, promoting the conversion of glucose into glycogen. Moreover, the antioxidant effect of microalgae will induce the breakdown or scavenging of free radicals and inhibit lipid peroxidation in rat tissue. *N. gaditama* particularly impacted glycemic control in the test rats, decreasing glycated hemoglobin levels. Microalgae restrict Lipoprotein lipase (LPL) activity, impeding the hydrolysis of triacylglycerides within the body. These organisms decrease oxidative markers responsible for catalyzing the formation of free radicals [32].

Microalgae such as *Chlorella* spp., *Nanochloropsis* sp., *Porphyridium* sp., and *Skeletonema* sp. emerged as potential antidiabetic agents by impeding the activity of the α-glucosidase with inhibition percentages of 12.5, 7.29, 12.65, and 11.26%, respectively, curbing the possibility of diabetes. α-glucosidase present on the surface of the intestinal membrane is essential in carbohydrate metabolism, breaking α-1,4-glucosidic bonds to produce glucose. Inhibition of this enzyme, for instance, by microalgal pigments including phycoerythrins, phycocyanins,

and allophycocyanins, reduces blood glucose levels [33]. Similarly, the n-hexane and ethanol extracts of *Chlorella vulgaris* were reported to inhibit α-glucosidase by 24.6% and 47.1%, respectively. Bioactive compounds such as flavonoids, phenolics, and steroids are capable of restricting this enzyme[34].

Table 2 Some microalgae used as antidiabetic agents.

No.	Species	Bioactive compounds	Actions	Reference
1.	*Chlorella* spp.	Phycoerythrins, phycocyanins, and allophycocyanins	Inhibiting the activity of the α-glucosidase enzyme	[33]
2.	*Chlorella vulgaris*	Flavonoids, phenolics, and steroids	Inhibiting the activity of the α-glucosidase enzyme	[34]
3.	*Diacronema lutheri*	Eicosapentaenoic and docosahexaenoic acids	Increasing insulin sensitivity	[35]
4.	*Nannochloropsis gaditana*	Not evaluated yet	Reducing pro-inflammatory cytokines, inducing insulin hormone secretion, inhibiting lipid peroxidation, reducing the activity of Lipoprotein lipase, and antioxidant	[32]
5.	*Nanochloropsis* sp.	Phycoerythrins, phycocyanins, and allophycocyanins	Preventing the activity of the α-glucosidase enzyme	[33]
6.	*Porphyridium* sp.	Phycoerythrins, phycocyanins, and allophycocyanins	Impeding the activity of the α-glucosidase enzyme	[33]
7.	*Skeletonema* sp.	Phycoerythrins, phycocyanins, and allophycocyanins	Restricting the activity of the α-glucosidase enzyme	[33]
8.	*Spirulina* sp.	ω-6 PUFA and phenolic compounds	Anti-hyperglycemic, anti-hyperlipidemic, and antioxidant	[36]
9.	*Spirulina* sp.	Insulin-like protein	Reducing blood glucose level	[37]

The ω-6 PUFA content synthesized by *Spirulina* sp. contributes to reducing blood glucose levels, accompanied by anti-hyperglycemic, anti-hyperlipidemic, and antioxidant properties. The PUFA stimulates antioxidant enzymes, such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), increasing their activity by 240% and 60% in healthy rats, as well as 19% and 59% diabetic in mice. Phenolic compounds present in the microalgae discussed in this paragraph can synergistically enhance enzyme activity [36].

Spirulina sp. also produces a protein characteristically similar to insulin, which functions properly at normal blood pH. This suggests its potential as a safer alternative to insulin treatment, avoiding immune resistance [37]. *Diacronema lutheri* is useful in insulin resistance management, hence incorporating this strain into diabetes-induced rats increases insulin sensitivity, preventing hyperinsulinemia associated with type 2 diabetes. This ability is attributed to eicosapentaenoic and docosahexaenoic acids, which counter obesity and other related problems [35].

Type 2 diabetes, a condition arising from pancreatic function deterioration, can be managed by microalgal antidiabetic compounds such as polyphenols and ω-3 PUFA. The provided antidiabetic activity involves regulating glucose metabolism within the body, specifically in blood vessels, and combating insulin resistance [38].

5. Bioprospecting of microalgae as antioxidant agents

Microalgal bioactive compounds are also essential in free radical scavenging as indicated in Table 3. *Chlorella vulgaris* and *Chlorella pyrenoidosa* usually produce phenolics and flavonoids as secondary metabolites. The methanol extract of *C. vulgaris,* containing the compounds, showed high free radical scavenging activity of 105.56% at a concentration of 1000 $\mu g/mL^{-1}$, with an LC50 value of 412.51 $\mu g/mL^{-1}$. Similarly, the methanol extract of C. pyrenoidosa demonstrated an LC50 value of 443.34 $\mu g/mL^{-1}$, along with 88.6% free radical scavenging at a concentration of 1000 $\mu g/mL^{-1}$ [39].

Table 3 Some microalgae used as antioxidant agents.

No.	Species	Bioactive compounds	Actions	Referenece
1.	*Botryococcus braunii*	Carotenoids and phenolics	Free radical scavenging	[40]
2.	*Chlamydomonas* sp.	Phenolics and flavonoids	Antioxidants	[41]
3.	*Chlorella*	Alkaloids, flavonoids, steroids, tannins, and saponins	Antioxidants	[42]
4.	*Chlorella pyrenoidosa*	Phenolics and flavonoids	Free radical scavenging	[39]
5.	*Chlorella* sp.	Phenolics and flavonoids	Antioxidants	[41]
6.	*Chlorella vulgaris*	Phenolics and flavonoids	Free radical scavenging	[39]
7.	*Chlorella vulgaris*	Carotenoids and phenolics	Free radical scavenging	[40]
8.	*Isochrysis* sp.	Carotenoids and phenolics	Free radical scavenging	[40]
9.	*Neochloris oleobundans,*	Carotenoids and phenolics	Free radical scavenging	[40]
10.	*Phaeodactylum tricornutum*	Carotenoids and phenolics	Free radical scavenging	[40]
11.	*Spirulina*	Phenolic compounds such as acacetin and pinocembrin, chlorogenic, gallic acid, taxifolin, kaempferol, apigenin, quercetin	Antioxidants	[36]
12.	*Spirulina* (*A. platensis*)	Saponins, quinines, glycosides, cardiac glycosides, terpenoids, triterpenoids, coumarins, steroids, steroids, phlorotannins, and anthraquinones.	Anticancer, antimicrobial, and antioxidant properties	[43]
13.	*Tetraselmis suecica*	Carotenoids and phenolics	Free radical scavenging	[40]

Spirulina produces a lot of antioxidants including phenolic compounds such as acacetin and pinocembrin, alongside PUFA which counters lipid peroxidation to prevent free radicals. Other phenolic compounds found in this strain are chlorogenic, gallic acid, taxifolin, kaempferol, apigenin, quercetin, and more, while the PUFA components encompass ALA, EPA, DHA, AA, GLA, and LA [36].

Spirulina (*A. platensis*) can be used as an anticancer, antimicrobial, and antioxidant agent. Furthermore, its crude extract contains saponins, quinines, glycosides, cardiac glycosides, terpenoids, triterpenoids, coumarins, steroids, steroids, phlorotannins, and anthraquinones. GCMS testing revealed several components including heptadecane, hexadecanoic acid, tributyl acetyl citrate, phytol, and other compounds [43].

Various microalgae strains such as *Tetraselmis suecica, Botryococcus braunii, Neochloris oleobundans, Isochrysis* sp., *Chlorella vulgaris*, and *Phaeodactylum tricornutum* exhibit antioxidant activity through carotenoids and phenolics which are essential in combating free radicals [40]. Additionally, *Chlorella* sp. methanol extract demonstrates high antioxidant activity due to its phenolics and flavonoids. *Chlorococcus* sp. extract. presents substantial radical scavenging activity, and *Chlamydomonas* sp. effectively inhibits free radicals, achieving 72% at a concentration of 100 $\mu g/mL^{-1}$ [41].

The alkaloids, flavonoids, steroids, tannins, and saponins components in *Chlorella sorokiniana* exhibit promising antioxidant potential [42]. Alkaloids, containing nitrogen atoms with available electron pairs, bind to free radicals, while flavonoids directly scavenge free radicals. These bioactive compounds chelate free radicals by donating hydrogen atoms or electrons. Additionally, they inhibit enzymes involved in free radical formation, such as xanthine oxidase, lipoxygenase, protein kinase C, cyclooxygenase, microsomal monooxygenase, mitochondrial succinoxidase, and NADPH oxidase [44,45].

The extensive potential of microalgae as antioxidant agents has been widely documented. For example, 100 µg of *Chlorella vulgaris*, *Desmococcus olivaceous,* and *Chlorococcum humicola* extracts demonstrated a radical scavenging activity of 68%, 62%, and 57%, respectively [46]. *Nannochloropsis oculata* extract produced terpenoids, carotenoids, and polyphenolics with free radical scavenging activity, presenting an LC50 of 52 $\mu g/mL^{-1}$, alongside bacterial and fungal growth inhibition with MIC values of 15.63-500 $\mu g/mL^{-1}$ [47]. Extracts from *Skletonema marinoi*, *Alexandrium andersoni*, and *Alexandrium andersoni* at a concentration of 100 $\mu g/mL^{-1}$ initiated a human melanoma cell viability below 20% [48]. Moreover, Fucoxanthin synthesized by microalgae inhibited enzymes associated with type-2 diabetes mellitus, including α-amylase and α-glucosidase, with respective values of 0.68 $mmol/L^{-1}$ and 4.75 $mmol/L^{-1}$ [49].

In the application of microalgae as medicines, ensuring safety remains paramount. Therefore, some studies suggested conducting in vivo toxicity tests, comprehensive biochemical analysis, and histological examinations on various organs, alongside determining appropriate dosages to assess potential effects. However, microalgae

usage as antidiabetic agents in appropriate doses has been reported to cause no vital organ damage [50] and avoid disruptions to the biochemical reactions of the body. To avoid potential toxin production, these organisms must be cultured under sterile conditions.

6. Conclusion

Microalgae are sources of functional medicines, particularly as anticancer, antioxidant, and antidiabetic agents. Various strains, such as *Arthrospira platensis* and *Chlorella pyrenoidosa* have been reported with capabilities of inhibiting proliferation, inducing apoptosis, and eliminating cancerous cells. As antidiabetic agents, *Chlorella vulgaris* and *Diacronema lutheri* have demonstrated the potential to inhibit α-glucosidase enzyme activity and enhance insulin sensitivity. *Chlorella, Isochrysis* sp., and *Neochlorosis oleobundans* produce bioactive metabolites including carotenoids and phenolic compounds useful in free radical scavenging. The versatile potential of these microorganisms presents an exciting avenue for green medicine. However, there is a need for more in-depth studies concerning their operating mechanisms and in vivo effectiveness. Optimization of microalgae applications for medicines production is still constrained by biomass provision. Large amounts of biomass are needed to meet industrial demand, therefore, the improvement of strains is essential to increase microalgal productivity.

7. References

[1] Andrade MKA, Lauritano C, Romano G, Ianora A. Marine microalgae with anti-cancer properties. Mar Drugs. 2018;16(5):165:1-17.

[2] Nigjeh ES, Yusoff FM, Alitheen MNB, Rasoli M, Keong YS, Omar AR Bin. Cytotoxic effect of ethanol extract of microalga, Chaetoceros calcitrans, and its mechanisms in inducing apoptosis in human breast cancer cell line. Biomed Res Int. 2013;2013:1-9.

[3] Samarakoon KW, Ko JY, Shah MMR, Lee JH, Kang MC, O-Nam K, et al. In vitro studies of anti-inflammatory and anticancer activities of organic solvent extracts from cultured marine microalgae. Algae. 2013;28(1):111-119.

[4] Suh SS, Yang EJ, Lee SG, Youn UJ, Han SJ, Kim IC, et al. Bioactivities of ethanol extract from the antarctic freshwater microalga, *Chloromonas* sp. Int J Med Sci. 2017;14(6):560-569.

[5] Somasekharan SP, El-Naggar A, Sorensen PH, Wang Y, Cheng H. An aqueous extract of marine microalgae exhibits antimetastatic activity through preferential killing of suspended cancer cells and anticolony forming activity. Evid Based Complement Altern Med. 2016;2016:1-8.

[6] Shady AAM, Gheda SF, Ismail GA, Cotas J, Pereira L, Karim AOH. Antioxidant and antidiabetic activity of algae. Life. 2023;13(2):1-26.

[7] Nasirian F, Dadkhah M, Kor MN, Obeidavi Z. Diabetes, metabolic syndrome and obesity: targets and therapy dovepress effects of *Spirulina* platensis microalgae on antioxidant and anti-inflammatory factors in diabetic rats. Diabetes Metab Syndr Obes. 2018:11:375-380.

[8] Ali MA, Rana K, Bhuiyan MSH, Miah S, Zahid S, Ahmed A, et al. Current status and prospects of microalgae bioactive com-pounds for anticancer and antiviral actions. Biomater J. 2022;1(2):28-36.

[9] Eseberri I, Zorita GS, Trepiana J, Arceo GM, Aguirre L, Laskibar MI, et al. Anti-obesity effects of microalgae. Int J Mol Sci. 2020;21(1):1-21.

[10] Abd El-Hack ME, Abdelnour S, Alagawany M, Abdo M, Sakr MA, Khafaga AF, et al. Microalgae in modern cancer therapy: current knowledge. Biomed Pharmacother. 2019;111:42-50.

[11] Ördög V, Stirk WA, Lenobel R, Bancířová M, Strnad M, Van Staden J, et al. Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. J Appl Phycol. 2004;16: 309-314.

[12] Matos J, Cardoso CL, Falé P, Afonso CM, Bandarra NM. Investigation of nutraceutical potential of the microalgae Chlorella vulgaris and Arthrospira platensis. Int J Food Sci Technol. 2020;55:303-312.

[13] Rickards RW, Rothschild JM, Willis AC, De Chazal NM, Kirk J, Kirk K, et al. Calothrixins A and B, novel pentacyclic metabolites from Calothrix cyanobacteria with potent activity against malaria parasites and human cancer cells. Tetrahedron. 1999;55(47):13513-13520

[14] Zhang J, Liu L, Ren Y, Chen F. Characterization of exopolysaccharides produced by microalgae with antitumor activity on human colon cancer cells. Int J Biol Macromol. 2019;128: 761-767.

[15] Tabarzad M, Atabaki V, Hosseinabadi T. Anti-inflammatory activity of bioactive compounds from microalgae and cyanobacteria by focusing on the mechanisms of action. Mol Biol Rep. 2020;47:6193-6205.

[16] Gupta SP, Siddiqi NJ, Khan HA, Alrokayan SH, Alhomida AS, Singh RK, et al. Phytochemical profiling of microalgae *Euglena tuba* and its anticancer activity in Dalton's lymphoma cells. Front Biosci. 2022;27(4):1-17.

[17] Dash S, Parida S, Sahoo B, Rath B. *In vitro* screening of antioxidant, antimicrobial and anticancer activities of cyanobacteria found across Odisha coast, India. Res Sq. 2022:1-19.

[18] Carté BK. Biomedical potential of marine natural products. Bioscience. 1996;46(4):271-286.

[19] Banker R, Carmeli S. Tenuecyclamides A-D, cyclic hexapeptides from the cyanobacterium *Nostoc spongiaeforme* var. *tenue*. J Nat Prod. 1998;61(10):1248-1251.

[20] Davidson BS. New dimensions in natural products research: cultured marine microorganisms. Curr Opin Biotechnol. 1995;6(3):284-291.

[21] Carmichael WW. Cyanobacteria secondary metabolites-the cyanotoxins. J Appl Bacteriol. 1992;72(6): 445-459.

[22] Moore RE. Cyclic peptides and depsipeptides from cyanobacteria: a review. J Ind Microbiol. 1996;16(2): 134-143.

[23] Ishiguro S, Uppalapati D, Goldsmith Z, Robertson D, Hodge J, Holt H, et al. Exopolysaccharides extracted from *Parachlorella kessleri* inhibit colon carcinoma growth in mice via stimulation of host antitumor immune responses. PLoS One. 2017;12:1-21.

[24] Fayyad RJ, Ali ANM, Hamdan NT. The specific anti-cancerous mechanisms suggesting *Spirulina* alga as a promising breast cancer fighter. Res J Pharm Technol. 2021;14(10):5599-5602.

[25] Prabhu PN, Ashokkumar P, Sudhandiran G. Antioxidative and antiproliferative effects of astaxanthin during the initiation stages of 1,2-dimethyl hydrazine-induced experimental colon carcinogenesis. Fundam Clin Pharmacol. 2009;23(2):225-234.

[26] Hosokawa M, Wanezaki S, Miyauchi K, Kurihara H, Konho H, Kawabata J, et al. Apoptosis-inducing effect of fucoxanthin on human Leukemia cell line HL-60. Food Sci Technol Res. 1999;5(3):234-246.

[27] Jahan S, Mahedi RA, Zaman F, Afrin S, Rodela A, Shimu S, et al. Amalgamation of astaxanthin and *Spirulina* may be a potential anti-carcinogenesis treatment strategy. Biomater J. 2022;1(1):18-22.

[28] Wang Z, Zhang X. Inhibitory effects of small molecular peptides from *Spirulina* (*Arthrospira*) platensis on cancer cell growth. Food Funct. 2016;7(2):781-788.

[29] Pungitore CR, Padrón JM, Leon LG, García C, Ciuffo GM, Martin VS, et al. Inhibition of DNA topoisomerase I and growth inhibition of human cancer cell lines by an oleanane from *Junellia* aspera (Verbenaceae). Cell Mol Biol. 2007;53(3):13-17.

[30] Lee SE, Lim JW, Kim H. Activator protein-1 mediates docosahexaenoic acid-induced apoptosis of human gastric cancer cells. Ann N Y Acad Sci. 2009;1171:163-169.

[31] Senousy HH, Abd Ellatif S, Ali S. Assessment of the antioxidant and anticancer potential of different isolated strains of cyanobacteria and microalgae from soil and agriculture drain water. Environ Sci Pollut Res. 2020;27(15):18463-18474.

[32] Wassila N, Zohra BAF, Hafida M, Ouahiba B, Samira B. Antihyperlipidemic and antioxidant effects of microalgae nannochloropsis gaditana in streptozotocin-induced diabetic rats. Rev Agrobiol. 2019;9(2): 1474-1483.

[33] Priatni S, Ratnaningrum D, Kosasih W. The screening of antidiabetic activity and the cultivation study of local marine microalgae. IOP Conf Ser Mater Sci Eng. 2021;1011:1-8.

[34] Ramdanawati L, Kurnia D, Roni A, Kalimatillah QA, Nurachman Z. Inhibition activities α-amilase of microalgae extract of chlorella vulgaris as candidate of antidiabetes. Indones Nat Res Pharm J. 2020;5(1): 105-113.

[35] Mayer C, Martine C, Ulmann L, Martin I, Zittelli GC, Faraloni C, et al. The potential of the marine microalga diacronema lutheri in the prevention of obesity and metabolic syndrome in high-fat-fed wistar rats. Molecules. 2022;27(13):1-18.

[36] Guldas M, Demirtas ZS, Sahan Y, Yildiz E, Gurbuz O. Antioxidant and anti-diabetic properties of *Spirulina* platensis produced in Turkey. Food Sci Technol. 2021;41(3):615-625.

[37] Anwer R, Al Shaqha WM, Fareed M. *Spirulina* as novel photosynthetic micro-algae for promising insulin-like protein other than pancreas. J Biotechnol Biomater. 2015;5(2):75.

[38] Bocanegra A, Gonzales MA, Garcimartin A, Benedi J, Muniz SFJ. Whole alga, algal extracts, and compounds as ingredients of functional foods: composition and action mechanism relationships in the prevention and treatment of type-2 diabetes mellitus. Int J Mol Sci. 2021;22(8):1-39.

[39] Raikar SM, Kalebar VU, Adhoni SA. Screening of pharmacological and cytotoxic activities of fresh water lake isolated microalgae chlorella vulgaris as-13 and chlorella pyrenoidosa as-6. Int J Bio Technology Res. 2018;8(4):1-8.

[40] Goiris K, Muylaert K, Fraeye I, Foubert I, De-Brabanter J, De-Cooman L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. J Appl Phycol. 2012;24(6):1477-1486.

[41] Adesalu TA, Temenu TO, Kuti FO, Adisa RA. Lipids composition and antioxidant properties of selected microalgae isolated from freshwater and soil samples. J Pharmacogn Phytochem. 2021;10(6):24-30.

[42] Mursandi H, Susanty D, Nurhayati L, Oksari AA. Short communication: antioxidant activity of ethanol extract of chlorella sorokiniana cultured in tofu wastewater. Nusant Biosci. 2022;14(2):55-59.

[43] El Din SMM, Hussein MH, Hamouda RA, Shehawy MA, El Maksoud AIA. Bioactive potentiality of some secondary metabolites extracted from microalga *Spirulina* platensis. J Chem Pharm Res. 2019;11(10):22-35.
[44] Banjarnahor SDS, Artanti N. Antioxidant properties of flavonoids. Med J Indones. 2014;23(4):239-244.
[45] Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82(4):513-523.
[46] Balaji M, Thamilvanan D, Vinayagam SC, Balakumar BS. Anticancer , antioxidant activity and GC-MS analysis of selected microalgal members of chlorophyceae. Int J Pharm Sci Res. 2019;8(8):3302-3314.
[47] Wali AF, Dhaheri Y Al, Pillai JR, Mushtaq A, Rao PGM, Rabbani SA, et al. LC-MS phytochemical screening, in vitro antioxidant, antimicrobial and anticancer activity of microalgae *Nannochloropsis oculata* extract. Separations. 2020;7(4):1-11.
[48] Lauritano C, Andersen JH, Hansen E, Albrigtsen M, Escalera L, Esposito F, et al. Bioactivity screening of microalgae for antioxidant, anti-inflammatory, anticancer, anti-diabetes, and antibacterial activities. Front Mar Sci. 2016;3:1-2.
[49] Kawee-Ai A, Kim AT, Kim SM. Inhibitory activities of microalgal fucoxanthin against α-amylase, α-glucosidase, and glucose oxidase in 3T3-L1 cells linked to type 2 diabetes. J Oceanol Limnol. 2019;37: 928-937.
[50] Habitri N, Belkacemi L. Antidiabetic effect of oral supplementation with *Caulerpa racemosa* powder. Eur J Biol Res. 2022;12(2):141-152.