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# Healthy Benefit of Microalgal Bioactive Substances

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**Abstract** Microalgae have been widely used as novel sources of bioactive substances. Along with this trend, the possibility of replacing synthetic preservatives with natural ones is receiving much attention. In general, microalgae are rich in various phytochemicals like carotenoids, phycocayanine, phenolics, amino acids, polyunsaturated fatty acids, and sulphated polysaccharides. These compounds are providing excellent various biological actions including, antioxidant, antimicrobial, antiviral, antitumoral, anti-inflammatory and anti-allergy effects. Their healthy benefit seemed to be due to different biochemical mechanisms. However, some microalgae species such as *Chlorella*, *Spirulina* and *Dunaliella* species have been used in several areas in nutraceutical, pharmaceutical, cosmetics, nutrition and functional quality of foods. In 2006, World Health Organization has been described *Spirulina* as one of the greatest super-foods on earth serving as an example of the potential of microalgae. This review provides background on current and future uses of microalgae as novel source of health promoting compounds.

***Keywords:*** *microalgae, antioxidant, nutrition, biological activities, functional food*

## 1. Introduction

Production of fine chemicals by microalgae has been known for decades and has now developed into an important industry. Some microalgae species have been used as food source for humans for over a thousand years [1,2]. *Chlorella* spp., *Spirulina* spp. and *Scenedesmus* spp. are the microorganisms that have recently drawn attention as commercially valuable sources of a wide spectrum of compounds including, high-quality protein, vitamins $B_{12}$, C and E, pigments (carotenoids and phycocyanin) and other bioactive chemicals, long chain polyunsaturated fatty acids especially ω-3 and ω-6 fatty acids, glycolipids, sulfolipids and other phenolic compounds [3,4,5]. While these are the species most often cited in the literature, it should be noted that other genus/ species are also being investigated as having commercial potential, e.g., *Prophyridum* spp. and *Dunaliela* spp. [6]. However, the main metabolites of potential commercial interest isolate from microalgae are listed in Figure 1.

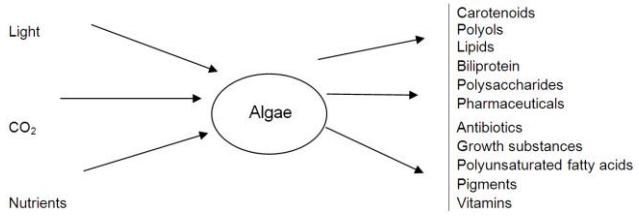



**Figure 1.** Diagrammatic representation of the microalgal process for bioactive substances

However, the Term "bioactive molecule" is a slang expression in common use and includes substances which may at low concentrations affect life processes - beneficial or harmful. Also, it refers to secondary metabolites that attract the attention of both scientists and industrialists. In spite of being potential producers of a wide spectrum of natural substances of vial human need, microalgae have so far been a rather under explored source in the development of biotechnology [7,8]. The major perceived advantage of microalgae over other organisms is that they are photoautotrophs and therefore do not require organic substances for energy: consequently, their large-scale culture is theoretically simpler and cheaper. Sunlight, water, $CO_2$ and inorganic nutrients are the basic requirement for algal growth. Furthermore, many algae also grown in saline to hyper-saline (3-33% w/v NaCl) waters, and thus do not compete with conventional agriculture for limited resources such as fresh water and arable land. This consideration is important to the production of large amounts of relatively low value chemical such as polysaccharides or some lipids and fatty acids feed-stocks for the chemical industry [9].

In general, the main advantages of cultivation of microalgae as a source of fine chemicals over conventional plants are summarizing as following:

1. Microalgal cultivation is an efficient biological system for use of solar energy to produce organic matter: indeed, algae grow faster than conventional plants throughout the year and produce the highest possible annual yield of biomass [1].
2. Microalgae can be grown well in hot desert climates, utilizing sea and /or brackish water, resources which is not suitable for conventional plants.
3. The life cycle of most microalgae is completed within several hours, which makes genetic selection and improvements in the species easy and fast.
4. Many species of algae can be induced to produce particularly high concentrations of compounds of

commercial interest such as protein, lipids, natural pigment and biopolymers [10,11,12].

5. Microalgae culture systems can produce up to 15,000 Kg of protein per acre per year. Whereas, soybeans typically produce less than 750 kg of protein per acre per year [13].

6. Microalgae cultivation does not contribute to soil erosion, requires little or no pesticides or herbicides, and requires a minimum of energy to cultivate and process.

7. Microalgae may be sun dried, heat dried, or freeze dried and extraction and purification of fine chemicals is possible in several different ways [14,15].

8. Microalgae can be grown in many countries using indigenous materials and without sophisticated technology, to produce healthy food-grad and fine chemicals [4].

9. Controlled experiments and anecdotal reports indicate that food-grade microalgae are nontoxic and may be beneficial sources of probiotic agent (term is refers to the promotion of life and health) such as polyunsaturated fatty acids especially ω-6 and ω-3 fatty acids [4]. The diagram showing the process followed in the search for bioactive compounds from microalgae is shown in Figure 2.

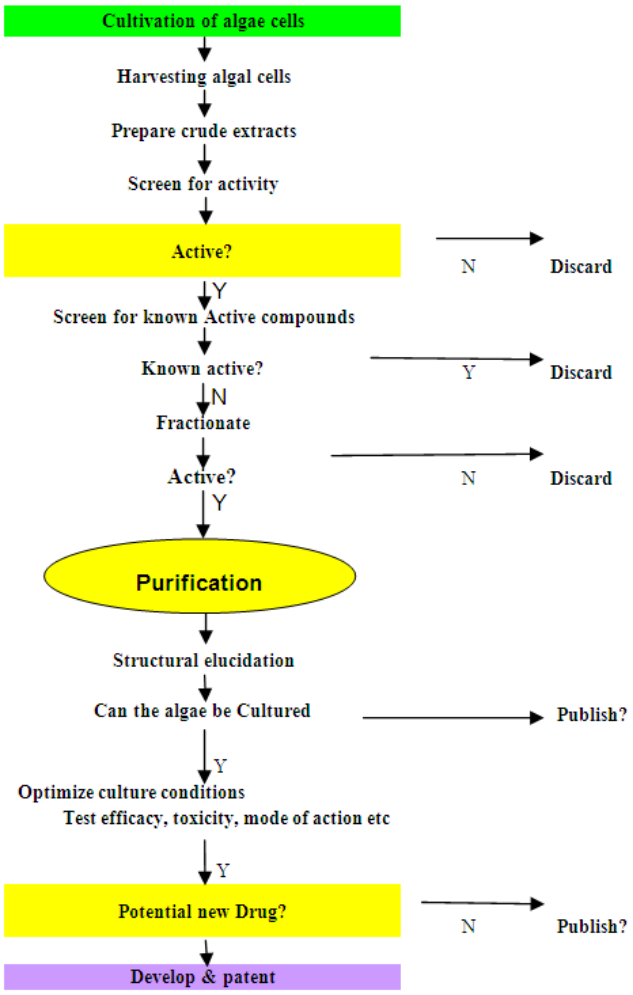



**Figure 2.** Flow diagram showing the process followed in the search for bioactive molecules from microalgae

Microalgae are still a relatively untapped resource, but selection of species and genetic manipulation which will lead to a high production of desired chemicals, coupled with improved technological processes should in the near future, create a new and promising biotechnological field. However, the searches in bioactive secondary metabolites of microalgae will continually provide novel useful and structurally specific compounds [7,16]. However, once a suitable microalga has been found before production of some bioactive chemicals by culture microalgae, some basic aspects should be outlined as following: (1) reliable long term culture of microalgae, (2) harvesting, (3) extraction, purification and identification of active compounds, (4) safety evaluation and (5) marketing. Finally, cultivation of microalgae using natural and manmade open–ponds is simple, but not necessary cheap due to the high downstream processing cost products of microalgae cultured in open-ponds could only be marked as value-added health food supplements, specially feed and bioactive agents [17].

**Antioxidants agents**

The most important reaction in lipid peroxidation is the autoxidation of unsaturated fatty acids. Autoxidation is known to proceed by a radical chain reaction formed from lipid (LH) then reacts with oxygen gas to form a lipid peroxyl radical ($LOO^{\cdot}$) which reacts with an additional lipid molecule (LH) to give a lipid hydroperoxide (LOOH) and lipid radicals ($L^{\cdot}$) in the propagation step. Then, lipid peroxyl radicals may combine to give non-radical compounds in the termination step.

Initiation: $LH \longrightarrow L^{\cdot}$

Propagation: $L^{\cdot} + O_2 \longrightarrow LOO^{\cdot}$

$LOO^{\cdot} + LH \longrightarrow LOOH + L^{\cdot}$

Termination: $LOO^{\cdot} + LOO^{\cdot} \longrightarrow$ non radical products

$LOO^{\cdot} + L^{\cdot} \longrightarrow$ non radical products

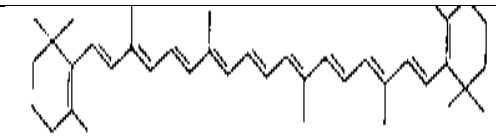
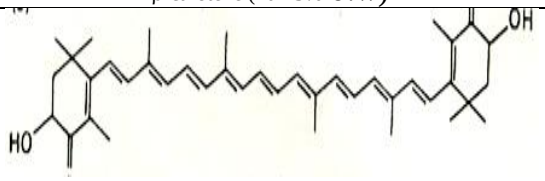
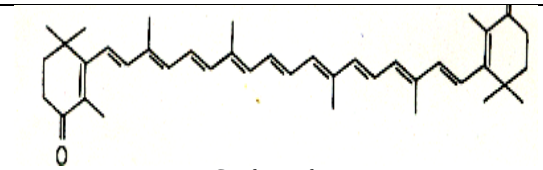
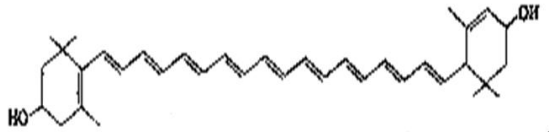
It is well known that active free radicals, including oxygen free radicals and non-oxygen free radicals, are by-products of normal metabolism. However, excessive free radicals are potential toxic hazards to various biological molecules through lipid peroxidation [18]. However, lipid peroxidation has attracted much attention in relation to oxidative damage of biological molecules, due to the formation of lipid hydroperoxides which in the presence of cellular iron containing compounds, can break down to yield oxygen radicals. Unsaturated fatty acids and cholesterol are easily oxidized, particularly in biological systems. The lipid peroxidation chain reaction yields a variety of mutagenesis, promoters and carcinogens such as fatty acids hydroperoxides, epoxoides and cholesterol hydroperoxides. The formation of these compounds in biological systems leads to a serious diseases such as ischemia-reperfusion injury, coronary arteriosclerosis and diabetes mellitus as well as being associated with aging and carcinogenesis [9,12,18]. Moreover, the lipid hydroperoxide can be decomposed to produce alkoxy (LO ) and peroxy radical (LOO ). They eventually yield numerous carbonyl products, which are responsible for DNA damage and generation of cancer and aging related diseases [19]. Also, lipid peroxidation is associated with wide variety of harmful effects including decreased membrane fluidity function, impaired hepatic, mitochondrial and Golgi apparatus function, inhibition of enzyme such as glucokinase, succinate dehydrogenase and

syntheytase, decomposed of some SH-compounds such as Co-enzyme A and inhibition of protein synthesis. It is commonly recognized that antioxidants can scavenge the harmful active free radicals in body cells and reduce the potential mutations. Microalgae are considered to be a non-conventional source of antioxidant, it contain tocopherols, phycocyanin, ascorbic acid, carotenoids and phenolic compounds [20,21]

## 2. Carotenoids

The carotenoids are lipid soluble pigments composed of isoprene units. They can be divided into two main groups: carotenes which include hydrocarbons only and the xanthophylls which are carotene derivatives containing epoxy, hydroxyl, ketonic, carboxylic, glycoside, allenic, or acerylene groups. The carotenoids function both as photoprotectants and, with varying degrees of efficiency, in trapping light energy for photosynthesis. All microalgae contain carotenoids**,** although each species usually contains 5 to 10 different major carotenoids (With a few minor components), the total number of carotenoids in all these classes of organisms is about 60. The structures of some carotenoids occur in most algal classes are shown in Table 1. These include β-carotene, astaxanthin, canthaxanthin and violaxanthin. The most advanced fine chemical production process using microalgae is the production of β-carotene from the green alga *Dunaliella salina*. This alga accumulated greater than 10% of its dry weight as β-carotene (as droplets in the chloroplast) making it the best eukaryotic source of this pigment [22,23]. Since, the relatively high price of about $2500 $Kg^{-1}$ of pure β-carotene, most of which is presently produced synthetically, thus the production of carotenoids from microalgal process even more attractive. The β-carotene from *D. salina* is a mixture of both the cis (>90%) and trans (>10%) isomers, unlike the synthetic β-carotene which is only the trans isomer. The natural β-carotene meets the requirements of some specialized markets (i.e., health food) [14].

**Table 1. Algae reported to accumulate significant quantities of carotenoids under certain conditions**

| Algae | Carotenoids | | Conditions |
|---|---|---|---|
| *Dunaliella salina* El-Baz, *et al.*, (2001) | β-carotene (10-13% d.w) |  β-carotene (10-13% d.w) | High salt, high light, low nitrogen |
| *Haematococcus Pluvialis* Steinbrenner and Hartmut (2001) | Astaxanthin (up to 4% d.w) |   Astaxanthin | N-stress |
| *Chlorella zofungiensis* Bar *et al.*,(1995) | β-carotene (50%of total car.) Canthaxanthin (25%of total car.) |   Canthaxanthin | High light +N-stress |
| *Spirulina spp.* Abd El-Baky *et al.*,(2003) | β-carotene Astaxanthin Lutein |   Lutein | High salt, high light, low nitrogen |

### 2.1. Factors Influencing Accumulation of Carotenoids in Algal Cells

A major criterion for the commercial cultivation of microalgae for carotenoids is that the algae has a high yield of the carotenoids of interest, and it is important to know which environmental and other factors enhance carotenoids production. For instance, β-carotene is a frequent constituent of the carotene mixture in microalgae but usually less than 1% of dry weight. However, in *Dunaliella* species, β-carotene is accumulated to up to 10% of the dry weight (d.w.) under high light intensity and conditions of limited growth such as high salinity, high temperature, or limited of phosphate, sulphate, iron, and nitrogen supplies [24]. Mojaat et al. [25] found that significant increase of cellular β-carotene content in *D. salina* culture enriched with $Fe^{2+}$ ions and organic source. β-carotene globules may protect *D. bardawil* against injury by the high intensity irradiation to which this algae in usually exposed [22].

#### 2.1.1. Effect of Nitrogen on Accumulation of Carotenoids in Algal Cells

Among the Chlorophyceae, nitrogen- limitation is clearly a major causal factor in the synthesis and accumulation of secondary carotenoids such as canthaxanthin and astaxanthin. For example, *C. zofingiensis*, C. *fusca* and *Haematococcus pluvialis* accumulate astaxanthin and sometimes also canthaxanthin, under conditions of nitrogen limitation [14,26]. The *Dunaliella salina* and *D. patva* accumulate large amounts of β–carotene under nitrogen limitation conditions [22,27].

#### 2.1.2. Mechanisms of Accumulation of Carotenoids under Nitrogen Starvation

Richmond [28] has been suggested that the dimerization of geranyl geranyl-pyrophosphate is the limiting step in carotenoids synthesis under nitrogen deficiency. Microalgae are known to alter their biosynthetic pathways due to changes in environmental conditions. Under nitrogen starvation, fat, carbohydrate and carotenoids were accumulated in algal cells and could reached up to 68%, 30% and 14% (d.w.), respectively [11,22]. The accumulation of these compounds was attributed to the fact that do not requires nitrogen for their synthesis and also, be the results of the fat, carbohydrate and carotenoids synthesizing enzymes being less susceptible to disorganization than in the system responsible for other compounds synthesis [23,29].

#### 2.1.3. Effect of Nitrogen Source on Accumulation of Carotenoids in Algal Cells

The form of the nitrogen source may influence in accumulation rate of secondary carotenoids in microalgae. *Haematococcus* accumulated astaxanthin rapidly if sodium acetate or glycine (amino acid) was supplied in the medium, whereas astaxanthin accumulation was delayed for some weeks if other organic or inorganic nitrogen sources were supplied [30]. Urea is the best nitrogen source for growing of *Chlorella* sp. and *Scendesmus* spp. to produce cell with high carotenoids contents. These algae can cleave urea with the aid of ATP: urea amido lyase other than enzyme of urease according to the overall equation [23,32].

$$\text{Urea+ATP} \xrightarrow{\text{UAL } Mg^{+}K} CO_2+2NH_3+\text{ADP+Pi}$$

However, *Chlorella* species can use ammonium nitrate salts as nitrogen source, but the pH of medium was changed with the growth of algae. Thus, change in pH of the culture depends on types of nitrogen source used in algal culture [31,32].

#### 2.1.4. Other Nutrients

Accumulation of secondary carotenoids has also been observed under conditions of phosphate, sulphate and iron limitation, and in fact, under set of conditions leading to the cessation of growth. The algae cell must, however, still have an adequate supply of carbon, either as $CO_2$ or as a suitable organic substrate [32].

### 2.2. The Influence of Other Environmental Factors

#### 2.2.1. Light

The increase of some active molecules such as carotenoids under high light intensity has been documented [24,33]. Under high irradiation the photosynthetic apparatus does not sufficient to utilize light energy, and the excess energy leads to the formation of highly active oxygen molecules. Therefore, the primary carotenoids cannot scavenge the radicals sufficiently, and additional mechanisms therefore are required for eliminating radicals or for reducing the illumination reaching the cell components under such conditions. In green microalgae such as *Chlorella* and *Haematococcus*, secondary carotenoids that accumulate in response to stress conditions may serve as agents protective against the effects of photooxidation [24].

#### 2.2.2. Temperature

Temperature has little effect on accumulation of carotenoids in microalgae. However, high temperature is a favorite condition to accumulation of high content of lutein, β-carotene and astaxanthin in some microalgae. Such temperatures are close to the edge of causing environmental stress. *D. salina* produced more β-carotene at high temperatures. *Haematococcus* spp. and *Chlorococcus* spp. accumulated high concentration of astaxanthin when grown at 25°C and 35°C, respectively [32].

#### 2.2.3. pH

Secondary carotenoids formation can also be influenced by pH. *Chlorella zofingiensis* dose not form secondary carotenoids under non-nitrogen limiting conditions at pH ranges 7 to 8, while large quantities of astaxanthin and canthaxanthin are formed at pH 5 to 6. At pH 9 and 8, *D. salina* and *Chlorococcus* sp. have great ability to produce high amounts of β–carotene and astaxanthin, respectively [32].

### 2.3. Bioactivities of Microalgae Carotenoids

#### 2.3.1. Antioxidant Activity

It is now evident that the antioxidant potential of carotenoids can significantly reduce free radicals and the oxidative lead to help the body maintain a healthy state. Carotenoids, especially β-carotene or astaxanthin, protect the living cells against oxidation by different mechanisms as follows: (1) quenching singlet oxygen and dissipating the energy as heat and (2) scavenging free radicals to prevent and terminate chain reaction. Due to its particular molecular structure, astaxanthin serves as an extremely powerful antioxidant. It has very effective quenching effect against singlet oxygen, a powerful scavenging ability for lipid and free radicals and effectively breaks peroxide chain reaction [4,12,34,35].

#### 2.3.2. Anticancer Activity

Epidemiological studies have demonstrated a correlation between carotenoid intake and the reduced incidence of coronary heart disease and certain cancers, macular degeneration, and increased resistance to viral, bacterial, fungal and parasitic infections [12,35,36]. Studies indicate that the mechanism for this protective attribute is partly due to the direct enhancement of the immune response by carotenoids. Anticarcinogenic effects of microalga *D. salina* as β-carotene of carotenoids are likely attributable to its antioxidant effect, in so far as oxygen radicals are related to the process of cancer initiation and propagation [37,38]. A synopsis of these studies demonstrates that supplementation with carotenoids increases the number of circulating lymphocytes (T-helper cells), enhances T and B lymphocyte proliferation, improves rejection of foreign tissue, increases killer cell destruction of tumor cells and neutrophil killing of *Candida* fungi, and inhibits loss of

macrophage receptors [39,40]. Tomita [41] found that mice fed on algal carotenoids had significantly reduced tumor growth when the primary lesion was excised and then re-challenged with the same tumor. Also, Tanaka [42] reported that astaxanthin is a possible chemopreventive agent for bladder carcinogenesis and such an effect is partly due to antioxidant effects and suppression of cell proliferation. Moreover, astaxanthin has been shown to reduce the carcinogenicity of aflatoxin by inducing enzymes called "CYP1A" and "CYP1A2" which enhance diversion of toxic byproducts towards detoxification pathways [43]. Astaxanthin may exert antitumor activity through the enhancement of particular immune responses which, elevated cytotoxic T lymphocyte (CTL) activity and interferon–γ (IFN-γ) production [44]. El-Baz et al. [23] reported that microalga *D. salina* and *Chlorella* sp. extracts rich in carotenoids content reduce the carcinogensis by pathway inducing the activity of glutathione S-transferase (GST) and other detoxification enzyme and non-enzyme systems. The neoxanthin and fucoxanthin were found to reduce cell viability through apoptosis induction in the human prostate cancer cells. These results suggest that ingestion of *Undaria pinnatifida* rich in neoxanthin and fucoxanthin might have the potential to reduce risk of prostate cancer [45]**.** An extract of *Spirulina* and *Dunaliella* algae were shown prevent tumor development in hamster buccal pouch when a solution was applied topically three times weekly for 28 weeks. The algae animals presented a complete absence of gross tumors [46].

#### 2.3.3. Immune Support of Algal Carotenoids

Singlet oxygen is also cytotoxic to the immune system by virtue of its ability to catalyze production of free radicals. This action can facilitate degradation of macrophage cell membranes resulting in dysfunction and reduced efficiency of phagocytosis [47]. Carotenoids have been shown to enhance both the non-specific and specific immune system and protect cell membranes and cellular DNA from mutation [39]. Carotenoids have a significant stimulatory effect on the immune system, as seen by the proliferative response of spleen cells and thymocytes during antibody response of mice. Astaxanthin enhances the release of interleukin-1 alpha and tumor necrosis factor alpha in mice greater than canthaxanthin and beta-carotene [48]. In Th1 cells, astaxanthin significantly enhanced the number of IgM antibody-secreting cells [40].

#### 2.3.4. Mechanism

Carotenods may modulate immune function by deactivating reactive chemical species such as free radicals, singlet oxygen and photochemical sensitzer. β-carotene, astaxanthin and other carotenoids functions as a chain-breaking antioxidant in a lipid environment, especially under low oxygen partial pressures as follow:

$$RH + \text{oxidant initiator} \longrightarrow R^{\cdot}$$

$$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$$

$$ROO^{\cdot} + \beta\text{-carotene} \longrightarrow \text{formation of inactive products}$$

The enhancement of immune function by carotenoids (e.g., canthaxanthin, astaxanthin), which do not possess provitamin A activity, strongly supports the antioxidant mode of action of carotenoids. Therefore, carotenoids may regulate immune cell function by protecting them against free radical-mediated genotoxic damage by modulating cell membrane fluidity, by increasing gap-junctional intercellular communication and by inhibiting arachidonic acid oxidation initiated by free radicals [49].

## 3. Phycobiliproteins

The major part of light energy used by any photosynthetic organism is harvested by a collection of accessory pigments, because chlorophyll-*a* absorb the light energy only in a limited region of the solar spectrum. In cyanobacteria the light-harvesting pigments are chlorophyll-a, carotenoids and phycobiliproteins. The latter are a group of intensely colored proteins. It comprises of a protein and chromophore, the protein moiety consists of α and β subunits of molecular weights in the range of 18 and 20 kDa each. The chromophore phycocyanobilin is a linear tetrapyrrole attached covalently to protein through a cysteine thioether bond. Phycocyanin content varies in the range of 10-15% of *Spirulina* biomass, based on the culture conditions [50]. Phycobiliproteins can be subdivided into three main groups according to their structure: phycocyanins (blue), allophycocyanin (blue) and phycoerythrins (red). Whereas phycocyanin and allophycocyanin are always present in Cyanophyceae and Rhodophyceae, phycoerythrin may be absent in the former. Phycobiliproteins are organized in supramolecular aggregates phycobilisomes in order to maximize energy transfer to the chlorophyll-protein complexes located at the thylakoid membrane [51,52].

### 3.1. Biological Effects of Phycobiliproteins

#### 3.1.1. Antioxidant Effects

Several investigator reported that phycocyanin, a water soluble biliprotein, is one of the major constituents of *Spirulina platensis* and *Spirulina maxima*, a blue green algae has significant antioxidant, anti-inflammatory, hepatoprotective, and radical scavenging properties [4,52]. It was suggested that the anti-inflammatory effect could be due to its ability to scavenge oxygen free radicals and inhibit enzymes involved in the formation of inflammatory prostaglandins. In fact, oxidative stress is considered one of the pathogenic factors of inflammation is implicated in oxidative stress-induced diseases. Phycocyanin contained an open chain tetrapyrrole chromophore known as phycocyanobilin (PCB) which is covalently attached to the apo-protein. PCB has chemical structure similar to that of bilirubin, a bile pigment, which is known to scavenge various reactive oxygen species *in vivo*. Bilirubin is also known to inhibit peroxnitrite ($ONOO^-$) mediated oxidations [52]. The C-phycocyanin from *S. platensis*, effectively inhibited $CCl_4$-induced lipids peroxidation in rat liver *in vivo*. All phycocyanin types significantly inhibited peroxyl radical-induced lipid peroxidation in rat liver microsomes and the inhabitation was dose dependent [4,8]. Phycocyanin of *Spirulina* spp. has scavenging activities against several radical species including superoxide, hydroxyl and alkoxy radicals [54]. Phycocyanobilin is responsible for the majority of the antioxidant activity of phycocyanin and may act as an

effective antioxidant in the living human body [55]. Abd El Baky and El-Baroty [4] found that water extract of *Spirulina* possessed significant scavenge activity toward hydroxyl and superoxide radicals. Moreover, the oral administration of phycocyanin could be used in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's, diseases brought on by oxidative stress-induced neuronal injury [56].

#### 3.1.2. Effects of Phycocyanin as Anticancer

Schwartz et al. [46] have shown that algae-derived phycocyanin had a cytostatic and cytotoxic activity against squamous cell carcinoma (human or hamster). Also, orally administration of *Spirulina* and *Dunaliella* extract (140 µg every 3 days for 28 days) prevented tumor development in hamster buccal pouches [57]. Carcinomas that were beginning to develop were destroyed in what appeared to be an immune response. The monocytes were found to be cytotoxic to tumor target cells *in vitro* and the lymphocytes were found to be T-cells. Thus the algae extract was believed to prevent cancer development by stimulating an immune response to selectively destroy small initial foci of developing malignant cells [5,56].

## 4. Vitamins

For human nutrition purposes, microalgae are considered to be a non- conventional source of vitamins, it contain several water and lipid–soluble vitamins. A number of vitamins are presented in higher concentrations in the microalgae than in conventional food, traditionally considered rich in vitamins such as vitamin E (tocopherols), C (ascorbic acid), β - carotene (pro-vitamin A) and $B_{12}$ [1,20]. Comparison with the content of vitamins C, $B_1$, $B_2$, nicotinic acid and folic acid in microalgae and higher plants which are natural and traditional sources of the vitamin, revealed that the microalgae exceeded the higher plants in their vitamins. However, algae like other plants, can synthesis most of vitamins. Some vitamins such as vitamin E, provitamin A and carotenoids have great market potential, especially for use as an antioxidant [9,12,14]. For instant, *Dunaliella* and *Chlorella* are rich in lipid soluble and B group vitamins [58]. *D. tertiolecta* is very rich in vitamin $B_2$, $B_{12}$, folic, C, nicotinic acid and E, which accounted 31.2, 0.7, 4.8, 163.2, 79.3, and 116.3 (mg/Kg d.w), respectively. Also, *C. stigmatophora* has a higher content of vitamin E, C, biotin, nicotinic acid and pantothenic acid than that conventional food traditionally considered rich in those vitamins. Thus, ingestion of relatively small quantities of *D. tertiolecta* and / or *C. stigmatophora* can cover the requirements for some vitamins in animal and human nutrition.

## 5. Polyunsaturated Fatty Acids

Several of microalgae, e.g., *Chlorella*, *Dunaliella* and *Spirullina* accumulate appreciable amount of fat (up to 60% of d.w.) when grown under certain environmental conditions, such as high in both temperature and light intensity and rise in salinity [59,33]. The algal lipids are known to contain a relatively high amount of long chain polyunsaturated fatty acids (PUFAs), especially omega (ω) 3- and 6- of FA series such eicosapentaenoic (EPA, 20:5 ω-3), docosahexaenoic (DHA, 22:6 ω-3), archidonic (AA, 20:4 ω-6), γ- linolenic (GLA, 18:3 ω-6) and α-linolenic (ALA, 18:3 ω-3) acid. These long chains PUFAs in algae have profound benefits and functions in dietetics and therapeutic uses [3,60]. They are believed to have a positive effect for the treatment of hypertension, premenstrual tension, various atopic disorders, diabetes, coronary heart disease, skin disease, hypertonia, cancer, hyperlipidemia, and number of the other cases [10]. In addition, GLA is the precursors of prostaglandins (E2 and F2) which possess potent vasodilators, antiflammatory and antiaggregatory properties as well as may be useful to correct defects occur in metabolism of essential fatty acid and imbalance of eicosanoids formation. Also, EPA plays an important role in mammals as an agent to prevent blood platelet aggregation [33,61]. DHA is important for the reception and transmission of impulses between brain cells [62]. Furthermore, dietary ω-3 PUFAs especially EPA and DHA are play a major role in modulating the biosynthesis of eicosanoids and in controlling the levels of blood lipids and lipoproteins. Thus, the principal clinical value of this fatty acid may be for the amelioration of atherogenesis and thrombogesis [16,63].

The correlation between lipid content and algal cell age, and nitrogen availability interact has been denominated [11,64]. N-starvation may increase or may leave unchanged the total lipid content depending on the dilution rate and cell age. Moreover, the effect may be quite different on the different lipid classes depending on cultures ages. The metabolic pathway for accumulation of fatty material lead to catabolism's of their compound throughout the β-oxidation to produce the excess of acetyl CoA. Thus the accumulation of many biological molecules such as carotenoids, tocopherol in alga cells cultivated under nitrogen starvation were increased, which acetyl CoA serve as precursors for synthesis of more organic substance as carbon and energy source [11,60]

## 6. Sulphated Polysaccharides

Sulphated polysaccharides (SPS) are a class of compounds containing hemi-ester sulphate groups in their sugar residues. These are commonly found in marine algae and higher animals, scarcely present in microbes and absent in higher plants. SPS are found in varying amounts in three major divisions of marine algal groups (Rhodophyta, Phaeophyta and Chlorophyta). SPS found in Rhodophyta are galactans consisting entirely of galactose or modified galactose units. They are known commercially as agar and carrageenan. Agar is composed of a backbone of alternating units of 3,6- anhydro-*α*-L-galactopyranosyl-(1→4) and O-*β*-D-galactopyranosyl-(1→3) and carrageenan is composed of only D-galactose units (1,3-linked *β*-D-galactose and 1,4-linked *α*-D-galactose). Carrageenan is classified into various types such as λ, k, ι, ε, μ, all containing 22-35% sulphate groups. In general, SPS of Phaeophyta are called fucans. This includes the compounds fucoidin, fucoidan, ascophyllan, sargassamn and glucuronoxylofucan. They comprise families of polydisperse heteromolecules based on L-

fucose, D-xylose, D-glucuronic acid, D-mannose and D-galactose [65].

### 6.1. Anticoagulant Activity of Sulphated Polysaccharides

The aqueous and hot water extracts from 45 species of microalgae have anticoagulant properties [65]. The hot water extract of *Monostroma nitidum* was found to be the most active among all algal tested; the active fraction was found to contain rhamnan sulphate. Also, water extract of *Codium latum* from Japan yielded sulphated arabinan (α-1→5) as an active molecule which gave about 12 times more anti-thrombin activity compared with heparin standard anticoagulant. Many reports also exist on anticoagulant activity of carrageenan. *Chondrus crispus* is the primary source of λ-carrageenan, whereas *Eucheuma cottoni* and *E. spinosum* are the sources of k- and i-carrageenans, respectively. Between the carrageenan types, λ carrageenan has approximately twice the activity of un-fractionated carrageenan and 4 times the activity of k-carrageenan. The anticoagulant activity of carrageenan appeared to be an anti-thrombic property. λ-carrageenan showed greater anti-thrombic activity than k-carrageenan probably due to its higher sulphate content. λ-carrageenan consistently prolonged the clotting time and was moretoxic than k-carrageenan.

### 6.2. Mechanism of Sulphated Polysaccharides as Anticoagulant

Anticoagulation occurs predominantly by inhibiting the key coagulation serine proteases, thrombin and factor Xa. This is facilitated by accelerating the activity of major physiological serine protease inhibitor-serpin antithrombin III (AT-III). There is lesser inhibition in the case of IXa, Xa, XIa, XIIa and kallikrein. Another serpin heparin co-factor II (HC-II) has been identified that exclusively inhibits thrombin, but has no significant activity against other coagulation or fibrinolytic proteases. The anti-haemostatic properties of heparin and other sulphated polysaccharides extend beyond anticoagulation and include fibrinolytic potentiation and anti-lipaemic effects [66]. Though heparin is a primary anticoagulant drug, it has some disadvantages like it is extracted from internal organs of higher animals and purified; hence its production is difficult and it exhibits haemorrhagic-like side effects. These disadvantages lead to research for discovering novel anticoagulant agents. Anticoagulant activity of green algal SPS has been assigned to the common pathway, primarily HC-II-mediated anticoagulant action. Investigations were conducted employing chromogenic substrates for the major coagulation enzymes, factor Xa and thrombin. These enzymes were inhibited indirectly by the algal SPS, however, via their potentiation of the activity of the serine protease inhibitors AT-III and HC-II. Also, the inhibition of thrombin by HC-II was potentiated by SPS [66,67].

### 6.3. Sulphated Polysaccharides as Antiviral

A novel sulfated-polysaccharide, calcium spirulan (Ca-SP) isolated from *Spirulina platensis*, that inhibits the replication *in vitro* of several enveloped viruses including Herpes simplex type I (HSV-1), human cytomegalovirus (HVMV), measles virus, mumps virus, influenza A virus, and HIV-1 virus [68]. The author added that the anti-HIV-1 activity of Ca-SP is comparable to that of dextran sulfate (DS, a known potent anti-HIV-1 agent), while its anti-HSV-1 activity were 4 to 5 times higher than that of dextran sulfate. The anti-HIV-1activity of Ca-SP or DS was 4 and 5 times higher in cultures treated with Ca-SP or DS during infection when compared with that in cultures treated with these substances after infection. Ca-SP was found to be superior to DS in possible therapeutic application because: (1) enhancement of viral replication at low concentrations, a usual phenomenon in DS, was not observed with Ca-SP, (2) Ca-SP was found to have a much lower anticoagulant effect than DS, (3) Ca-SP was found to have a much longer half-life in the blood of mice compared to DS, and (4) Ca-SP was 4 to 5 times more effective in inhibiting HSV-1 compared to DS [7]. Also, aqueous extract of *S. platensis* inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC), and *Langerhans* cells [69].

## 7. Microalgae as Growth Promoters

The potential application of microalgae is in the area of microbial culture and tissue in industrial applications has been reported by Kay [1]. Aqueous extracts of green microalgae such as *Chlorella* and *Secnedesmus* have been shown to stimulate the growth and yield of yeasts and other microorganisms. For example, the hot-water extract of *S. obliquus* resulted in 3 times greater yields of *Rhizobium japonicum* compared to yeast extract. The potential market for growth promoting substrates for the fermentation industry is extremely large. Hot-water extract of *Chlorella* also promote growth of plant tissue and cell culture. However, extract of *Scendesmus* and *Spirulina* as a replacement for serum was used in animal cell tissue culture growth medium. The market for serum for this purpose is very large; these algal extracts may prove to be an excellent alternative [28].

Algal extracts are also known to stimulate the growth of plants and this could be due, in part, to the presence of auxins, gibberellins, cytokinins and other hormones. Natural ethylene releasing chemical 1-amino-cycloproane-1-carboxylic acid from algae, which may be responsible for some the plant growth stimulating effect are well documented. The possible application of microalgal extracts in horticulture and agriculture has yet to be explored [28]. Abd El Baky et al. [35] found that the treated wheat plants irrigated with seawater (10% and 20% levels) with algal extracts led to increase content of antioxidant compounds such as vitamin C, carotenoids, tocopherols and phenolic acids in both leaves or grains and improve all growth parameter. Thus, increases in antioxidant content in wheat cells grow under salt stress condition seem helpful in protection against ROS induced by salt stress [21].

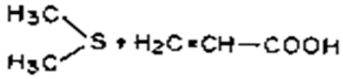
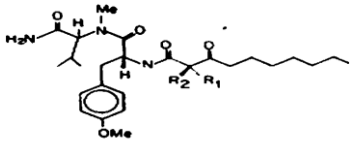
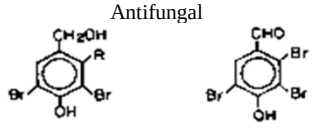
## 8. Microalgae with Pharmaceutical Impact

### 8.1. Antimicrobial Effect

In the last decade, the screening of microalgae, for antibiotics and pharmacologically active compounds has received ever increasing [4,70]. A large number of antibiotics compounds, many with novel structures, have been isolated and characterized. Also, many cyanobacteria have been shown to produce antiviral and antimicrobial compounds. Specifically, the antimicrobial activity of the methanolic extract of *S. platensis* was explained by the presence of γ-linolenic acid, present in a high concentration in this alga [71]. Several, fatty acids had been reported to have some antimicrobial activity, specifically palmitoleic and oleic acids it was hypothesized that lipids kill microorganisms by leading to disruption of the cellular membrane(s). The susceptibility of Gram-negative bacteria to killing by lipids was notable [72,73] and is probably due to the differences in the outer membrane or the cell wall of bacteria. However, a wide range of antimicrobial activities have also been observed with extracts of microalgae. The *Chlorella* spp., *Scenedesmus* spp., *Chlamydomonas* spp., *Euglena viridis*, *F. ambigua* and *Microcystis aeruginosa* have been reported as the main groups of microalgae to produce antimicrobial substances [74]. Several of the bioactive compounds may find application in human or veterinary medicine or in agriculture. Others should find application as research tools or as structural models for the development of new drugs. The microalgae are particularly attractive as natural sources of bioactive molecules since these algae have the potential to produce these compounds in culture which enables the production of structurally complex molecules which are difficult or impossible to produce by chemical synthesis [14,15,75]. Antimicrobial (antibacterial, antifungal, antiprotozooal) substances identified include fatty acids, glycollipds, acrylic acid phenolics, cyclic peptides, N-glycosides, sulphate-polysaccharides,β-diketone, isonittrile-containing indole, alkaloids such as haploindole and various toxins such as nodularin, goniasutoxin, saxitoxin, okadaic acid and ciguatoxin [60]. The structure of some bioactive compounds isolated from different algal spices is shown in Figure 3.

**Production of biologically active substances by algal culture**

Several studies have shown that the production of the active compounds depended on the growth phase and/or culture conditions and this means that culture conditions for the production of bioactive compounds must be optimized [76]. Nutrient limitation, especially N and P limitation has been shown to be necessary for the production of high levels of acutiphycin in *Oscillatora acutissima* and maximum concentrations are achieved in early stationary-phase cultures. Addition of N, P, or organic carbon greatly reduces the formation of acutiphycin. On the other hand, antibiotic production in *Nostoc muscorum* and *Scytonema* spp. was most affected by the N and Fe content of the medium and was enhanced in actively growing cultures. Also, the production of toytoxin in *Scytonema ocellatum* is unusual for a secondary metabolite in that it is produced throughout the cell cycle [77]. Culture studies with the domoic acid (DA) producing *Nitzschia pungens* forma multiseries showed that DA was produced at a rate of 1pg DA $cell^{-1}$ $d^{-1}$ only during the log and stationary growth phases in presence of N source and light [78]. Other environmental factors may also be important, for instance, the antibiotic cyanobacteria, in LU1 from *N. linckia* is synthesized throughout the growth cycle at low temperatures [79]. Similarly lipid production is enhanced by silicon starvation, and low temperatures generally enhance the content of long-chain polyunsaturated fatty acids such as EPA in many algae [80]. However, the physiological control of secondary metabolite formation in algae is not extremely limited due to limited information available about the biosynthetic pathways. Thus, many studies are necessary to use genetic engineering and recombinant DNA technology for increase the production of bioactive compounds by either developing over-producing strains [81].

| Algae | Compounds | References |
|---|---|---|
| | Sulfur compounds | |
| *Phaeocystis poucht* |  Acrylic acid Gram-negative organisms | Richmond (1986 ) |
| green algae *Chara globularis* |  | Richmond (1986 ) |
| Blue green alga *Lyngbya mjuscula* *L. gracilis* |  $R_1$ = H , $R_2$ = Me $R_1$ = Me, $R_2$ = H | Richmond (1986 ) |
| *Calothrix brevissima* | Antifungal  R = H R = Br | Richmond (1986 ) |

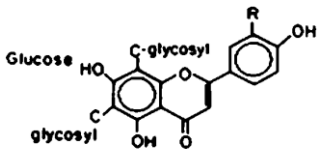
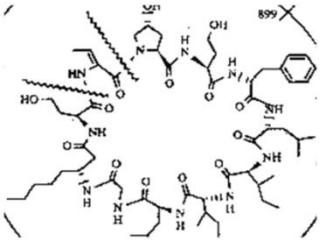
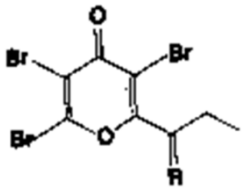
| | | |
|---|---|---|
| Chlorophyta<br>*Nitella hookeri* |  <br>R =H<br>R =OH<br>Antifungal | |
| Blue green alga<br>*Hormothamnion Enteromorphoides* |  <br>Hormothamnin A<br>Antimicroal to *Bacillus subtills* | Gerwick *et al*. (1992) |
| *Delisea fimbriata* | Cytotoxic to several cancer cell lines<br>  <br>Gram-negative organisms<br>R= Br or O | Baker and Josph (1984) |

**Figure 3.** Antibiotic compounds isolated from algal strains

## 8.2. Antiviral Effect

Many studies have demonstrated that microalgae compounds could efficiently inhibit the growth of various viral species and the mechanism of the inhibition has been elucidated [16,21]. Ohta et al., [82] found that herpes simplex virus (HSV-1) was inhibited by methanolic extracts of *D. bioculata* C-523, *D. primolecta* C-525. *Lyngbya* spp. M-9 and *Lyngba aerugino-coerulea* M-12. *D. primolecta*, had the highest anti-HSV-1 activity, since 10µg $ml^{-1}$ of extract was completely inhibited the viral growth. This activity was similar to that of acyclovir (antiviral agent) at the same concentration.Three pheophorbide-like compounds having anti-HSV activity were isolated from the algal *D. primolecta* and the viral growth completely inhibited at concentration of 5mg/ml [60]. Gustafson et al. [83] found that sulfonic-acid-containing glycolipids isolated from *L. lagerheimii* and *Ormidium tenue* had antiviral active against HIV-1 cultured in human lymphoblastoid CEM cell lines.

## 8.3. Probiotic Effects

Tsuchihashi et al. [84] found that an intake of *Spirulina* at 5% of the diet increased the population of *Lactobacillus* in the caecum of rats by 3 times over a control group of rats not fed *Spirulina*. Similar results were obtained by De-Mule, [85] in *in vitro* studies with *L. lactis* and *Candida albicans*. Parada et al. [86] have reported a stimulatory effect of extra-cellular products from algae on lactic acid bacteria including *L. lactis*, *Streptococcus thermophilus*, *L. casei*, *L. acidophilus*, and *L. bulgaricus*. In humans, *Lactobacillus* is believed to have three functions: to improve digestion and absorption of foods, to protect from infection, and to stimulate the immune system. In patients with an Acquired Immune Deficiency Syndrome (AIDS), nutrient malabsorption associated with opportunistic infections from microorganisms like *C. albicans* can speed expression of disease symptoms [7].

# 9. Other Healthy Benefits

## 9.1. Hypolipidemia Effect

Many studies had conducted on lipid and cholesterol-regulatory of microalgae. The reduction of serum cholesterol by *Spirulina* was well documented. Kato et al. [87] found that the elevation of total cholesterol, LDL+VDL cholesterol, and phospholipids was reduced significantly in serum of rats fed on high cholesterol diet supplemented with 16% *Spirulina*. The fall in HDL cholesterol caused by the high cholesterol diet was also reduced in mice fed the high cholesterol diet in the presence of *Spirulina*. Adipohepatosis induced by a high fat and high cholesterol diet was also reduced rapidly when the mice were shifted from the high fat, high cholesterol diet to a basal diet supplemented with *Spirulina*. De Rivera et al. [88] found that rats fed on diet supplemented with 5% *Spirulina* and either 60% glucose or 60% fructose had lower levels of hepatic triglycerides and phospholipids compared to control groups. The concentration of hepatic triglycerols and cholesterol in rats treated with single intraperitoneal dose of (1ml/kg) of $CCl_4$ were significantly decreased after rats fed on basal diet supplemented with *Spirulina* [67]. In a human clinical study on 15 diabetic patients, a significant reduction in total lipid, free fatty acid, triglyceride levels and LDL/HDL ratio were observed [89]. However, *C. pyrenoidosa* has ability to prevent hyperlipidemia and atherosclerosis in chronic high-fat fed rats and hamsters

and could be potential in use to prevent intestinal absorption of redundant lipid [90].

## 9.2. Effects against Diabetes and Obesity

Hypoglycemic natural products comprise flavonoids, xanthones, terpenoids, glycosides peptides, polysaccharide and other. The water extracts of freshwater algae *O. limnetica*, *B. ganeshii* and *M. lacustris* exhibited effect similar to the well know sulfonylurea drug like butamide [91]. Also, the water-soluble fraction of *Spirulina* had lowering effect on fasting serum glucose level in diabetic animals, while the water-insoluble fraction suppressed glucose level at glucose loading [92]. In diabetic's patient, a significant decrease in the fasting blood sugar level was observed after 21 days of 2 g/day *Spirulina* supplementation [89]. Becker et al. [93] have found that a supplementary diet of 2.8 g of *Spirulina* 3 times $d^{-1}$ over 4 weeks resulted in a significant reduction of body weight in obese out-patients. *Spirulina* has also been found to suppress high blood pressure in rats [94]. A vasodilating property of rat aortic rings by *Spirulina* possibly dependent upon a cyclo-oxygenase-dependent product of arachidonic acid and nitric oxide has been reported by Paredes-Carbajal et al. [94]. Cheng-Wu et al. [96] studied the effect of polysaccharides and phycocyanin on peripheral blood and hematopoietic system of bone marrow in mice. Their studies showed that C-phycocyanin and polysaccharides from *Spirulina* had a high erythropoetin (EPO) activity. Rodriguez-Hernandez et al. [97] found the administration of *Spirulina maxima* to both female and male diabetic mice prevent the accumulation of trigacylglycerol in the liver.

## 9.3. Radiation Protection Effect

The radio-protective effect of a crude ethanol precipitate (CEP) of *Spirulina platensis* was studied using the micronucleus test in polychromatic erythrocytes (PCE) of mouse bone marrow. CEP extract caused a significant reduction of micronucleus frequencies induced by γ-radiation. γ -radiation followed by treatment with CEP led to about the same radio-protective effect as CEP treatment followed by γ-radiation. This compound probably acts as a DNA-stabilizing factor, and they ruled out the possibility of a radical scavenging mechanism [98]. These results reflect the ability of CEP to act as antimutagenic and repair-stimulating capacities [46]. Mazo et al. *[99]* exposed rats to γ-radiation and followed intestinal barrier permeability to polyethylene glycol 4000. Addition of *Spirulina* to the diet led to near complete normalization of permeability. Also, feeding phycocyanin extract from *Spirulina* to rats exposed to x-rays (5 Gy) resulted in the normalization of decreases in dehydrogenase activity, energy-rich phosphate level, and efficiency of antioxidant defense observed in rats without phycocyanin supplementation [100].

## 9.4. Chemopreventive Effect of Algae

Chemoprevention is defined as the use of biological or chemical agents (natural or synthetic to reveres, suppress, or prevent carcinogenic progression of invasive cancer is promising anticancer approach aimed at reducing morbidity and mortality. The chemoprevention action can be separated into two main categories, depending upon whether they act extracellular or intercellular. The extra cellular activities occur mainly during the preparation of foods and in the intestine, while the intracellular activities may occur in cells of different organs. Antioxidant is one of the mechanisms that can occur in both categories [101]. Moreover, there are other mechanisms, more specific or site oriented, that can complement the total beneficial potentials of chemo-preventers [102]. However, a brief summary of the available information regarding the mechanism of action of chemopreventers as anticarcinogens, or as preventers of other chronic diseases is presented.

### 9.4.1. Inhibition of Carcinogen Formation

A number of chemopreventers were found to inhibit in situ formation of carcinogenic nitrosamines, produced from secondary amines and nitrite in an acidic environment of the stomach. Formation of these nitrosamines is prevented or reduced by ascorbic acid or phenolic compounds [102,103].

### 9.4.2. Inducing Agents

There are enzymatic systems involved in reducing the level of mutagenic/carcinogenic species in the body, usually by oxidative detoxification system. Microalgae contained several compounds can induce or enhance the activity of enzymatic systems known to detoxify carcinogens (e.g., glutathione S-transferase, GST), these compounds act as chemopreventers. Algal components that could act as chemopreventers by this type of mechanism are carotenoids, tocopherols and ascorbic acid [34].

### 9.4.3. Scavenging Agents

Components in microalgae that can physically react with the electrophilic forms of carcinogens, can trap some carcinogens making a conjugate with them, thereby eliminating them as potential carcinogens. Examples of this type of chemopreventers are phenolic compounds, vitamins (C, E) and flavonoids. Also, several algal extract *Dunaliella*, *Chloroella* and *Spriulina* act as chemopreventive agent, which increase the level of glutathione as non-enzymes detoxification system [34,52].

### 9.4.4. Suppressing Agents

Algal extracts possess beneficial effects in suppressing different steps in metabolic pathways required for development of tumors. This category of chemo-preventers may react on a number of systems or processes involved in tumor promotion/ progression, usually by inhibiting certain metabolic processes. For instance, they may inhibit arachidonic acid metabolism, activity of protease, polyamine metabolism, or protein kinase C. They also may inhibit the oxidative DNA damage induced by different promoters and/or carcinogens. Microalgae content some antitumorigenic components, such as phytoestrogen and phycocanine [104].

### 9.4.5. Chemopreventers and Coronary Heart Disease

An oxidative change in LDL is one of the early phases in the development of atherosclerosis. Recently, a hypothesis has been proposed that LDL, which has

undergone oxidative damage, is considerably more atherogenic than native LDL. Oxidized LDL is taken up more readily than native LDL by macrophages to create the cholesterol-loaded foam cells, which, as fatty streaks, are characteristic for the atherosclerotic lesions. Chemopreventers with algal antioxidant capacity, such as vitamin C and E, carotenoids, or phenolics, were found to block the oxidative modification of LDL, thereby preventing the atherosclerotic changes in the blood vessels [34]. Antioxidants, especially vitamin E as a lipid soluble substance increased the resistance of LDL to oxidation. Thus, thereby prevents or reduces the formation of the coronary heart diseases. Microalgal is richest organisms in Folic acid and other B groups. However, low intake of dietary folate is responsible for the high levels of homocysteine in the blood, which in turns promote formation of oxidized LDL and atherogenesis [101].

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