ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Bio- Prospecting Microalgae For Value Added Products.

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ABSTRACT

Microalgae are autotrophs and are the major producer in aquatic system. Microalgae in order to survive in the varying conditions they produce metabolites which were found to have potential biological activity. Microalgae or bio compounds of the microalgae were found to have appreciable biological activities which enable them to find application as pharmaceutical agent, nutraceutical agent, antioxidant agent, anticancer agent, antiviral agent etc. Microalgae are considered to be nutritious since they have more proteins, carbohydrates, lipids, vitamins etc. and some microalgae have been used as food and feed for animals. Increased lipid content in few prokaryotic and eukaryotic microalgae enable them to find application in the production of biodiesel. In this review, the various application aspects of microalgae were approached.

Keywords: Microalgae, bioactivity, biodiesel, value added products.

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ISSN: 0975-8585

INTRODUCTION

Microalgae are simple, microscopic photosynthetic organisms, and can exist in fresh water as well as in marine water. They can be a prokaryotic or eukaryotic organism. The most commonly exploited microalgae are Cyanophyceae (Blue green algae), Bacillariophyceae (diatoms), Chlorophyceae (Green algae), Chrysophyceae (Brown algae) [1]. They produce a wide range of metabolites such as carbohydrates, lipids, proteins, pigments, vitamins, minerals etc. These metabolites were found have potential activities such as antibacterial, antifungal, antiviral, antioxidant, antioxidant, neuro protective activities. Some of the microalgae have lipid content in excess and are exploited for biodiesel production. Due to the presence of more nutrients such as protein, carbohydrate and lipid, they are used as food and feed for animals. Microalgae act as a nutraceutical agents as PUFA, vitamins and minerals are produced by microalgae. In recent decades the microalgae become interesting biological factories and are cultivated in large scale due to their commercial applications.

MICROALGAE AS FOOD AND FEED

Microalgae are known to have high protein, carbohydrate, lipids, vitamins, PUFA, carotenoids contents. Due to their high nutritional value some of the microalgae have been used as food and feed. Large scale cultivation of microalgae for their use as food and feed has been practiced since ancient time. Some algae like *Chlorella, Spirulina, Nostoc, Aphanizomenon flos-aquae* have been consumed as food owing to their rich nutritional value.

Since ancient times, *Spirulina* has been harvested and used as food by Mexicans and Africans [2]. In mexico, *Spirulina* is used for making Tecuitlatl, a dry cake. In Chad, Dihe, a dry cake made using *Spirulina* [3]. Nowadays, *Spirulina* is cultivated in large scale and used as protein supplement in Asian countries suchas Thailand, Korea, China and Japan. *Spirulina* is considered as a nutritious food due to its high content of proteins, α-linolenic acid, vitamins, minerals and pigments. *Spirulina* is used as a prebiotic food in order to induce the growth of intestinal microflora [4, 5, 6].

Nostoc, a cyanobacteria has also been used as food by Chinese [7]. *Nostoc flagelliforme* and *Nostoc sphaeroides* are regarded as a healthy food by Chinese. These algae have also find a way used in Chinese traditional medicine for the treatment of hypertension, hepatitis and diarrhea [3].

Another microalga that is considered as nutritious food is *Chlorella*. It contain proteins, carotenoids, vitamins and β-glucan [8]. β glucan acts as an antioxidant and reduces the blood lipid level [9]. *Chlorella vulgaris* is consumed as food and used in drink supplements [10, 11, 12]. Most of these microalgae are produced in the form of pills, capsules and powder.

FEED

Microalgae are also used as feed for fish, farm animals due to their high content of carbohydrates, lipids, proteins, vitamins, minerals and essential fatty acids. They improve the immunity and fertility of animals. Most commonly used feeds are *Chlorella, Tetraselmis, Pavlovalutheri*, *Isochrysis, Pluerochrysiscarterae*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis* [4, 13, 14, 15, 10].

MICROALGAE AS SOURCE OF PIGMENTS

The photosynthetic organisms are the primary producers which are capable to convert the light energy into chemical energy with the help of organic pigments. The microalgae contain three major classes of pigments- cholorophylls, carotenoids and phycobilins. These pigments have been found to have potential biological activities. Organic pigments isolated from microalgae are reported to be non-toxic and non-carcinogenic [16]. They are used as food colorants in ice creams, chewing gum, soft drinks, alcoholic beverages, desserts, sweet cake decoration, milk shakes. Since the pigments are degradable and can be recycled, it can be used as an alternate source of chemical ink used in the printing.

Carotenoids

Carotenoids are a group of fat soluble natural pigments, constitute a class of terpenoids, which are derived from 8 isoprene molecules and contain 40 carbon atoms. Carotenoids are classified into carotenes (which contain only hydrocarbons) and xanthophylls (oxygenated derivatives of carotenes) [17]. The xanthophylls pigments commonly produced by microalgae are violaxanthin, antheraxanthin, zeaxanthin, neoxanthin, lutein, loroxanthin, astaxanthin and canthaxanthin [18].

Diatom produces Diatoxanthin, diadinoxanthin and fucoxanthin [18]. Luetin and zeaxanthin plays a major role in maintaining normal vision and they occur naturally in retina [19]. These two pigments have the ability to reduce the risks of cataract, macular degeneration and atherosclerosis [20]. Some of the microalgae such as *Chlorella zofingiensis, Chlorella protothecoides, Chlorella vulgaris, Scenedesmus almeriensis, Haematococcus pluvialis, Chlorella saccharophila* [21] have been identified as major source of zeaxanthin, β-carotene, lutein, zeaxanthin and astaxanthin pigments.

β- *Carotene*

β-Carotene is an important fat soluble pigments of carotenoids family. β- Carotene acts as a pro-vitamin A and in the human system this precursor gets converted into vitamin A. *Dunalina salina* is the main source of β-carotene pigment. β- Carotene exhibit antioxidant property; it scavenges the harmful oxy radical and reduces the accumulation of free radicals. It inhibits the oxidation of low density lipoprotein and reduces the cholesterol and high density lipoprotein level [22]. It was noted that it delays the development of atherosclerosis [23].

Astaxanthin

Astaxanthin acts as antioxidant, photoprotectant and has ability to reduce the age related visual impairment and blindness such as AMD (age related macular degeneration) and cataracts. Astaxanthin acts as protective agent against oxidation of essential polyunsaturated fatty acids, UV light effects, cancer and immune response. It modulates the humoral and non-humoral immune response. It acts as an inhibitor of colon cancer cell line by decreasing the expression of Cyclin D1 and increase the expression of p53 [24]. Major microalgae sources of astaxanthin are *Haematococcus pluvialis* and *C.zofingiensis* [25].

Phycobilin

Phycobilin is water soluble pigment found in cyanobacteria. Three major types of phycobilin are phycocyanin, allophycocyanin and phycoerythrin. *Spirulina* and *Porphyridium* are considered to be the major sources of the phycobilin. Phycobilin pigments are used as food colorant and are used in chewing gum, ices creams, posicles, candies, soft drinks and dairy products [26]. They can also be used as fluorescent biomarkers in biotechnological diagnostic tools. Phycoerythrin is a red coloured pigment. The major source of phycoerythrin is *Porphyridium cruentum*. Phycocyanin is a blue coloured pigment derived from blue green algae with the major source being *Spirulina plantensis*. Phycocyanin is a potential pharmaceutical applicant due to the antioxidant property [27].

MICROALGAE AS A SOURCE OF PHARMACEUTICAL COMPOUNDs

Microalgae produce metabolites in order to survive in varying condition of salinity, temperature, pressure etc. These metabolites are usually secondary metabolites, which can be a carbohydrate, proteins, enzymes, pigments, antibiotics and some were found to be toxic compounds. These metabolites were found to have pharmaceutical property such as antiviral, antimicrobial, antifungal, neuroprotective products, therapeutical compounds etc.

Toxic metabolites

Some of the cyanobacteria and dinoflagellates produce toxic metabolites and cause harmful algal blooms in aquatic environment. These toxins can cause serious hazards to other organisms including higher organisms like human, cattle etc. Phycotoxin, a toxic metabolite of few microalgae, Cyanobacteria and

Dinoflagellate, causes neurotoxicity, cytotoxicity, hepatoxicity and dermal toxicity [28] on humans and animals. Some fresh water microalgae like *Microcystis*, *Anbaena*, *Oscillatoria* and *Nostoc* are regarded as a common producers of toxins like microcystins, homo and anatoxin, saxitoxins. Some *Dinoflagellates* such as *Alexandrium*, *Dinophysis*, *Karenia* and *Gymnodinium* species produce toxins which cause shellfish poisoning [3].

These toxic metabolites are found to have potential biological activities like anticancer, antibacterial, antibiotic and can be exploited as a promising drug compound. The phycotoxin has shown to have anticancer, antifungal activity [29]. Okadaic acid, a neurotoxin derived from *Dinophysis*, is used in the treatment of cognitive impairment and schizophrenia [30]. *Spirulina* is used to treat health problems such as diabetes, arthritis, anaemia, cardiovascular disease and cancer [31]. *Spirulina platensis, Spirulina maxima* are known to produce compounds that have neuroprotective activity. *Chlorella* contains β -glucan which acts as an immunostimulator and reduce the blood lipids level [9].

Anticancer agent

Pectentoxins produced by *Dinophysis* has anticancer activity on cancer cell lines [32]. Macrolide amphidinolides [33] of *Amphidinium* and curacin A [34] of *Lyngbyamajuscule* have been proved for its anticancer activity. Cryptophycin, a compound isolated from Blue green algae, *Nostoc* [35] is reported to have anticancer activity. Saxitoxin and brevetoxins, polyketide neurotoxins of dinoflagellates are found to have anticancer property. Scytophycins of *Scytonema pseudohofmanni* is found to have anticancer activity [36]. Scytonemin of *Stigonema* inhibits the enzyme protein serine/ threonine, can be used as an antiproliferative and anti-inflammatory agent [37](Stevenson *et al*., 2002).

Exopolysaccharides

Exopolysaccharides of few microalgae were identified to have potential biological activity. They act as antioxidants, antiviral, anticancer, anti-inflammatory agents, etc. *In vitro* studies using extracts of some of the microalgae, *Spirulina plantesis, Spirulina maxima* [38, 39, 40, 41], *Porphyridium sp.* [42, 43], *P. cruentum* [44, 45], *P. purpureum* [38](Radonic *et al*., 2010), *R. reticulate* [43] have been found to have antiviral activity. Exopolysaccharide of *Rhodella reticulate* [46] have shown antioxidant activity. Exopolysaccharide of *Porphyridium*, *Chlorella stigmatophora* and *Phaeodactylum tricornutum* has shown anti-inflammatory activity [47, 48, 49].

MICROALGAE AS SOURCE OF NUTRACEUTICAL AGENT

Some food which acts as pharmaceutical and nutritional agent falls under nutraceutical compound. Some of the nutraceutical agent are omega 3 polyunsaturated fatty acids (PUFA), carotenoids like astaxanthin, β -carotene. Eicosapentaenoic Acid (EPA) and decosahexaenoic acid (DHA) are associated with prevention and treatment of several diseases such as atherosclerosis, thrombosis, arthritis, cancers etc.

Polyunsaturated fatty acids

Fatty acids are long chain hydrocarbons with carboxylic group [50](Rubio-Rodriguez, 2010). On the basis of hydrocarbon, Fatty acids are classified into saturated and unsaturated fatty acids. Polyunsaturated fatty acids are long chain unsaturated fatty acids. PUFA are a vital nutrient for humans as it cannot be produced by our system. Therefore, it has to be obtained through diet [51, 52]. Major source of PUFA is cod liver oil of fish. But fish obtain this nutrient by consuming the microalgae, major producer in water system. The polyunsaturated fatty acids are known to have the ability to cure arthritis, obesity, schizophrenia, neurodegenerative diseases such as parkinson's disease, multiple sclerosis, heart disease and atherosclerosis [53, 54].

Omega 3 (PUFA n-3) and omega 6 (PUFA n-6) are the derivative of PUFA. Eicosapentaenoic acid (EPA, C20:5, n-3) and Docosahexaenoic acid (DHA, C22:6, n-3) are derivative of omega 3 fatty acid. Omega-3 are present in food as α-linolenic acid (ALA, C18:3, n-3). α -linolenic acid is converted into EPA and DHA in our body. Since this conversion is inefficient and limited [55], these two are obtained from diet. DHA is a vital nutrient for the fetal brain development and for optimal retinal function in newborns [56, 57](McCann and

Ames, 2005, Medina *et al.,* 1999). EPA has the ability to lower the blood cholesterol level and prevents the coronary heart disease. EPA is isolated from *Porphyridium cruentum* and *Monodus subterrans* [51], *Nitzschia inconspicua* [58]. DHA is isolated from *Schyzochytrium sp*. [3]. Omega 6 fatty acids are also present in the food as linoleic acid (LA, C18:2, n-6). Arachidonic acid (AA, C20:4, n-6) is a derivative of omega 6 fatty acid [55]. Arachidonic acid acts a precursor of prostaglandin and leucotrienes with important role in circulatory and central nervous system [59]. *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Porphyridium cruentum* [60],*Crypthecodinium cohnii* [61]are also the best source of linoleic and arachidonic acid. *Nannochloropsis gaditana*, *Monoraphidium brauni, Scenedesmus obtusiusculus, Pseudodictyosphaerium jurisii, Choricystis minor, Nannochloropsis limnetica* are some of the sources of PUFA.

MICROALGAE AS AN ALTERNATE BIOENERGY SOURCE

Biodiesel is produced by transesterification of fatty acid of vegetable oil and animal fats. This oil consists mainly of triglycerides. In transesterification, the triglycerides are converted to methyl esters by means of methanol in the presence of catalyst. This process occurs in three steps: triglycerides are converted to diglycerides, then to monoglycerides and finally glycerol and methyl esters. The catalyst used in the transesterification can be alkali, acid [62, 63] or lipase [64]. Most commonly used alkali catalysts are sodium or potassium hydroxide. Sodium methoxide, an Alkoxide is a better catalyst than the sodium hydroxide [65].

Recently microalgae have been used as an alternative source for biodiesel production due to the presence of the high percentage of lipids. The advantage of using the microalgae for biodiesel production over the vegetable oil and animal fat is that they are fast growing compared to the terrestrial plants; easy to cultivate and extract the lipid throughout the year; yield is more when compared to plant. Further, it will not affect the world food price [65]. Microalgae being autotrophic, they absorb the CO_2 from the atmosphere and release the oxygen. So there is possibility of reducing the CO_2 level in the atmosphere.

Microalgae accumulate the lipids in the form of neutral lipids mostly triglycerides which are stored in cytosol [66, 67]. These neutral lipids can be extracted and used for the biodiesel production. Some of the fresh and marine microalgae which are reported to have neutral lipids are *Chlorella vulgaris, Crypthecodinium cohnii, Cylindrotheca, Dunaliella salina, Isochrysis sp., Nannochloris sp, Nannochloropsis oculata, Neochlorisoleo abundans, Nitzschia sp, Phaeodactylum tricornutum, Porphyridium cruentum, Schizochytrium sp., Tetraselmis suecica, Botryococcus braunii, Scenedesmus obliquus, Skeletonema coastatum, Chaetoceros muelleri* [68, 69].

The lipid content of the microalgae can be altered by varying the culture and nutrient conditions. Nutrient starvation is commonly used for increasing the lipid yield. Most commonly nitrogen, phosphorus and silicon nutrient limitation yielded more neutral lipid in *Stephanodiscus minutulus* [70], *Chlamydomonas moewusii* [71], *Cyclotella cryptica* [72], *Scenedesmus sp.*[73, 69, 74, 75]. Change in the temperature has significantly increased the lipid content of *Nannochloropsis salina* [76], *Isochrysis galbana* [77]. *Dunaliella salina* [78], *Nitzschialaevis* [79], have shown increased lipid yield when the salinity was increased.

CONCLUSION

Microalgae are known to have wide range of applications due to presence of high value added metabolites such as pigments, vitamins, carbohydrates, minerals, proteins etc. They are known to produce compounds having antioxidant activity, antiviral activity, antifungal activity, anticancer activity which can serve as a lead molecule for drug development. The increased nutrient contents in microalgae makes them a potential source of nutraceutical compounds such as PUFA, carotenoids, vitamins, minerals. Another advantage of microalgae is their use in biodiesel production and being autotrophic they can used as carbon dioxide sequestrant that can decrease the carbon dioxide load in the environment.

REFERENCES

[1] Qiang Hu, Milton S, Eric J, Maria G, Matthew P, Michael S and Al Darzins. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The Plant Journal 2008; 54: 621–639.

[2] Barsanti L, Gualtieri P. Algae: anatomy, biochemistry and biotechnology. Florida: Taylors R Francis Group, 2006; pp301.
[3] Wan-Loy Chu Biotechnological applications of microalgae. IeJSME 2012: 6 (Suppl 1): S24-S37.
[4] Yamagushi K. Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: A review. J. Appl. Phycol. 1997; 8: 487–502.
[5] Liang, S, Xueming L, Chen F, Chen Z. Current microalgal health food R & D activities in China. Hydrobiologia 2004; 512: 45–48.
[6] Vilchez C, Garbayo I, Lobato MV, Vega JM. Microalgae-Mediated chemicals production and wastes removal. Enzyme Microb. Technol. 1997; 20: 562–572.
[7] Han D, Bi Y, Hu Z. Industrial production of microalgal cell-mass and secondary products – species of high potential. Nostoc. In: Richmond A, ed. Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell Science, 2004: 304-311.
[8] Becker W. Microalgae in human and animal nutrition. In: Richmond A, ed. Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell Science, 2004: 312-351.
[9] Iwamoto H. Industrial production of microalgal cell-mass and secondary products – major industrial species. Chlorella. In: Richmond A, ed. Handbook of microalgal culture: biotechnology and applied phycology. UK: Blackwell Science, 2004: 255-263.
[10] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 2004; 65: 635–648.
[11] Rasmussen, R.S.; Morrissey, M.T. Marine biotechnology for production of food ingredients. Adv. Food Nutr. Res. 2007; 52: 237–292.
[12] Raposo, M.F.J.; Oliveira, S.E.; Castro, P.M.; Bandarra, N.M.; Morais, R.M. On the utilization of microalgae for brewery effluent treatment and possible applications of the produced biomass. J. Inst. Brew. 2010; 116: 285–292.
[13] Apt KA, Behrens PW. Commercial developments in microalgal biotechnology. J. Phycol. 1999; 35: 215–226.
[14] Müller-Feuga A. The role of microalgae in aquaculture: situation and trends. J. Appl. Phycol. 2000; 12: 527–534.
[15] Borowitzka MA. Microalgae for aquaculture: Opportunities and constraints. J. Appl. Phycol. 1997; 9: 393–401.
[16] Joshi VK, Devender A, Anju B, shashi B: Microbial pigments. Indian J. of Biotechnology 2003; 2: 362-369.
[17] Del Campo AJ, García-González M, Guerrero MG. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Appl. Microb. Biotechnol. 2007; 74: 1163–1174.
[18] Eonseon, J, Polle JEW, Lee HK, Hyund SM, Chang M. Xanthophylls in microalgae: from biosynthesis to biotechnological mass production and application. Microb. Biotechnol. 2003, *13*, 165–174.
[19] Alves-Rodrigues A, Shao A, The science behind lutein, Toxicology Letters 2004;150 (1): 57-83.
[20] Roberts RL, Green J, Lewis B, Lutein and zeaxanthin in eye and skin health. Clin. Dermatol. 2009; 27, 195–201.
[21] Dilip S, Munishi P, Serena W, Anshu SM, Deepak KT, Colin JB, Characterization of a new zeaxanthin producing strain of *Chlorella saccharophila* isolated from New Zealand marine waters, Bioresource Technology2013; 143: 308–314.
[22] Shaish A, Harari A, Kamari Y, Cohen H, Schonfeld G, Harats D. Application of *Dunaliella* in atherosclerosis. In: Ben-Amotz A, Polle JEW, Rao DVS, eds. The alga *Dunaliella*: biodiversity, physiology, genomics and biotechnology. USA: Jersey Science Publishers, 2009: 475-494.
[23] Phang SM, Chu WL, Wong CY, Teoh ML, Tan KP, Lee HK. A checklist of microalgal isolates from Ny Alesund, Svalbard. Polarnet Technical Report: the Journal of the CNR's Network of Polar Research, 8th Ny Alesund Seminar, Cambridge, UK, 16 – 17 October, 2007, 2008; 1–14.
[24] Palozza P, Torelli C, Boninsegna A, Simone R, Catalano A, Mele MC, Picci N. Growth-inhibitory effects of the astaxanthin-rich alga *Haematococcus pluvialis* in human colon cancer cells. Cancer Lett. 2009; 283: 108–117.
[25] Ip PF, Chen F. Production of astaxanthin by the green microalga *Chlorella zofingiensis* in the dark. Process Biochem. 2005; 40: 733–738.
[26] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioengrg 2006; 101: 87-96.
[27] Bhat VB, Madyastha, KM. C-phycocyanin: a potent peroxyl radical scavenger in vivo and in vitro. Biochemical and Biophysical Research Communications. 2000; 275 (1): 20 – 25.

[28] Carmichael WW. Health effects of toxin-producing cyanobacteria: the CyanoHabs. Hum Ecol Risk Assess 2001; 7: 1393-1407.
[29] Garcia-Camacho F, Gallardo-Rodriquez J, et al. Biotechnological significance of toxic marine dinoflagellates. Biotechnol Adv 2007; 25: 176-194.
[30] He J, Yang Y, Xu HY, Zhang X, Li XM. Olanzapine attenuates the okadaic acid-induced spatial memory impairment and hippocampus cell death in rats. Neuropsychopharmacology. 2005; 30: 1511-1520.
[31] Mani UV, Iyer UM, Dhruv SA, Mani IU, Sharma KS. Therapeutic utility of *Spirulina*. In: Gershwin ME, Belay A, eds. *Spirulina* in human nutrition and health. Boca Raton: CRC Press 2008: 71-99.
[32] Jung JH, Sim CS, Lee CO. Cytotoxic compounds from the two sponge association. J Nat Prod 1995; 58: 1722-1726.
[33] Kobayashi J, Shimbo K, Kubota T, Tsuda M. Bioactive macrolides and polyketides from marine dinoflagellates. Pure Appl Chem 2003; 75: 337-342.
[34] Burja AM, Abou-Mansour BEB, Payri C, Burgess JG, Wright PC. Culture of marine cyanobacterium, *Lyngbya majuscule* (Oscillatoriaceae) for bioprocess intensified production of cyclic and linear lipopeptides. J Microbiol Meth 2002; 48: 207-219.
[35] Schwartz RE, Hirsch CF, Sesin DF, Flor JE, Chartrain M, Fromtling RE. Pharmaceuticals from cultured algae. J Ind Microbiol 1990; 5: 13-123.
[36] Ishibashi M, Moore RE, Patterson GML, Xu C, Clardy J.Scytophycins, cytotoxic and antimycotic agents from the cyanophyte *Scytonema pseudohofmanni*. J Org Chem 1986; 51: 5300-5306.
[37] Stevenson CS, et al. The identification and characterization of the marine natural product scytonemin as a novel antiproliferative pharmacophore. J Pharmacol Exp Ther 2002; 303: 858-866.
[38] Radonic A, Thulke S, Achenbach J, Kurth A, Vreemann A, König, T, Walter C, Possinger K, Nitsche A. Anionic polysaccharides from phototrophic microorganisms exhibit antiviral activities to Vaccinia virus. J. Antivir. Antiretrovir. 2010; 2: 51–55.
[39] Hayashi T, Hayashi K, Maeda M, Kojima I. Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J. Nat. Prod. 1996; 59: 83–87.
[40] Hayashi K, Hayashi T, Kojima I. A natural sulphated polysaccharide, calcium spirulan, isolated from *Spirulina platensis*: In vitro and ex vivo evaluation of anti-herpes simplex virus and anti-human immunodeficiency virus. AIDS Res. Human Retrovir. 1996; 12: 1463–1471.
[41] Hernandez-Corona A, Nieves I, Meckes M, Chamorro, G, Barron BL. Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. Antivir. Res. 2002; 56: 279–285.
[42] Huleihel M, Ishanu V, Tal J, Arad SM. Activity of Porphyridium sp. polysaccharide against Herpes simplex viruses in vitro and in vivo. J. Biochem. Biophys. Methods. 2002; 50: 189–200.
[43] Huleihel M, Ishanu V, Tal J, Arad SM. Antiviral effect of the red microalgal polysaccharides on Herpes simplex and Varidella zoster viruses. J. Appl. Phycol. 2001; 13: 127–134.
[44] Huang J, Chen B, You W. Studies on separation of extracellular polysaccharide from *Porphyridium cruentum* and its anti-HBV activity in vitro (in Chinese). Chin. J. Mar. Drugs 2005; 24: 18–21.
[45] Fabregas J, García D, Fernandez-Alonso M, Rocha AI, Gómez-Puertas P, Escribano JM, Otero A, Coll JM. In vitro inhibition of the replication of viral haemorrhagic septicaemia virus (VHSV) and African swine fever virus (ASFV) by extracts from marine microalgae. Antivir. Res. 1999; 44: 67–73.
[46] Chen B, You B, Huang J, Yu Y, Chen W. Isolation and antioxidant property of the extracellular polysaccharide from *Rhodella reticulata*. World J. Microbiol. Biotechnol. 2010, 26, 833–840.
[47] Guzman S, et al. Anti-Inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phytother. Res. 2003; 17: 665–670.
[48] Matsui SM, Muizzudin N, Arad SM, Marenus K. Sulfated polysaccharides from red microalgae anti-inflammatory properties in vitro and in vivo. Appl. Biochem. Biotechnol. 2003; 104: 13–22.
[49] Arad SM, Atar D. Viscosupplementation with algal polysaccharides in the treatment of arthritis. WIPO Patent WO/2007/066340, 14 June 2007.
[50] Rubio-Rondriguez N, Beltran S, Jaime I, De Diego S, Sanz MT, Carballido SM. Production of omega-3 polyunsaturated fatty acid concentrates: A review., Innovative Food Science and Emerging Technologies, 11, hal. 2010; 1-12.
[51] Ward OP, Singh A. Omega-3/6 fatty acids: Alternative sources of production., Process Biochemistry, 2005; 40, hal. 3627–3652.
[52] Handayania NA, Ariyantib D, and Hadiyanto H, Potential Production of Polyunsaturated Fatty Acids from Microalgae, Internat. J. of Sci. and Eng. 2011; 2(1): 13-16.
[53] Kerby NW, Stewart WDP. The biotechnology of microalgae and cyanobacteria. In L.J. Rogers and J. R. Gallon, Biochemistry of the algae and cyanobacteria. Oxford: Clarendon Press. 1987.

[54] Danielle S, Robert B and Shaker A. Mousa. Omega-3 Fatty Acids EPA and DHA: Health Benefits Throughout Life. American Society for Nutrition. Adv. Nutr. 2012; 3: 1–7.
[55] Kalogeropoulos N, Chiou A, gavala E, Christea M, Andrilkopoulos NK.), Nutritional evaluation and bioactive microconstituents (carotenoids, tocopherols, sterols and squalene) of raw and roasted chicken fed on DHA-rich microalgae, Food Research International, 43, hal.2010; 2006–2013.
[56] McCann JC, Ames BN. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. The American Journal of Clinical Nutrition, 2005; 82 (2): 281-295.
[57] Medina AR, Cerda´n LE, Gime´nez AG, Pa´ez BC, Gonza´lez MJI, Grima EM. Lipase-catalyzed esterification of glycerol and polyunsaturated fatty acids from fish and microalgae oils., Journal of Biotechnology 1999; 70: 379–39.
[58] Chu WL, Phang SM, Goh SH. Environmental effects on growth and biochemical composition of Nitzschia inconspicua Grunow. J Appl Phycol 1996; 8: 389-396.
[59] Medina AR, Grima EM, Gime´nez AG, Gonza´lez MJI. Downstream Processing Of Algal Polyunsaturated Fatty Acids., Biotechnology Advances. 1997; 16: 517-580.
[60] Ana O, Digna G and Jaime F. Factors controlling eicosapentaenoic acid production in semicontinuous cultures of marine microalgae. Journal of Applied Phycology 1997; 9: 465–469.
[61] Jiang Y, Chen F, Liang SZ. Production potential of docosahexaenoic acid by the heterotrophic marine dinoflagellate *Crypthecodinium cohnii*. Process Biochem 1999; 34: 633-637
[62] Fukuda H, Kondo A, Noda H. Biodiesel fuel production by transesterification of oils. J Biosci Bioeng 2001; 92: 405–16.
[63] Meher LC, Vidya Sagar D, Naik SN. Technical aspects of biodiesel production by transesterification — a review. Renew Sustain Energy Rev 2006; 10: 248–68.
[64] Sharma R, Chisti Y, Banerjee UC. Production, purification, characterization, and applications of lipases. Biotechnol Adv 2001;19: 627–62.
[65] Chisti Y. Biodiesel from microalgae. Biotechnology Advances. 2007; 25: 294–306.
[66] Day JG, Benson EE and Fleck RA. 1999. In vitro culture and conservation of microalgae: application for aquaculture, biotechnology and environmental research. In Vitro Cellular & Developmental Biology. 1999; 35: 125-136.
[67] Hu Q, Sommerfeld M, et al. Microalgal triacylglycerols as feedstock for biofuel production: Perspectives and advances. Plant Journal.2008; 54: 621-639.
[68] Mcginnis KM, Dempster TA and Sommerfeld MR. Characterizaion of the growth and lipid content of the diatom *Chaetoceros muelleri*. J. Appl. Phyco. 1997; 9: 19-24.
[69] Illman AM, Scragg AH and Shales SW. Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. Enzyme and Microbial Technology.2000; 27: 631-635.
[70] Lynn SG, Kilham SS, Kreeger DA, Interlandi SJ. Effect of nutrient availability on the biochemical and elemental stoichiometry in the freshwater diatom *Stephanodiscus minutulus* (bacillariophyceae). J. Phycol. 2000; 36: 510–522.
[71] Arisz SA, et al. Polar glycerolipids of *Chlamydomonas moewusii*. Phytochemistry 2000; 53: 265–270.
[72] Miao X, Wu Q. Biodiesel production from heterotrophic microalgal oil. Bioresour. Technol. 2006; 97: 841–846.
[73] Xin L, Hong-ying H, Ke G, Ying-xue S. Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus sp*. Bioresour. Technol. 2010; 101: 5494–5500.
[74] Lv JM, Cheng LH, Xu XH, Zhang L, Chen HL. Enhanced lipid production of *Chlorella vulgaris* by adjustment of cultivation conditions. Bioresour. Technol. 2010; 101: 6797–6804.
[75] Griffiths M, Harrison S. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J. Appl. Phycol. 2009; 21: 493–507.
[76] Boussiba, S, Vonshak A, Cohen Z, Avissar Y, Richmond A. Lipid and biomass production by the halotolerant microalga *Nannochloropsis salina*. Biomass 1987; 12: 37–47.
[77] Zhu C, Lee Y, Chao T. Effects of temperature and growth phase on lipid and biochemical composition of *Isochrysis galbana* tk1. J. Appl. Phycol. 1997; 9: 451–457.
[78] Azachi M, Sadka A, et al. Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant alga *Dunaliella salina*. Plant Physiol. 2002; 129: 1320–1329.
[79] Chen GQ, Jiang Y, Chen F. Salt-induced alterations in lipid composition of diatom *Nitzschia laevis* (bacillariophyceae) under heterotrophic culture condition1. J. Phycol. 2008; 44: 1309–1314.