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Review Article

Bioactive Compounds from Green Microalga – *Scenedesmus* and its Potential Applications: A Brief Review

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ABSTRACT

Microalgae biomass has been recognised to have great potential as a source of novel bioactive compounds with industrial as well as health promoting applications in human, animal and aquatic lives. Several species of microalgae have undergone various screenings to identify and tap into these valuable resources, among them are the species of the genus *Scenedesmus*. Although it is more commonly known as a source of food for herbivorous zooplankton and in biofuel production because of its high lipid content, *Scenedesmus* has exhibited the potential of being a source of high-value compounds with antibacterial properties. These antibacterial activities have a wide range of applications in various industries that have not been broadly explored and fully exploited. This review aims to briefly cover four decades of research done on bioactive compounds, nutritional composition, biotechnological applications and antibacterial activities in *Scenedesmus* spp.

Keywords: *Scenedesmus*, bioactive compounds, biotechnological applications, antibacterial inhibition

INTRODUCTION

Freshwater microalgae are widely distributed in rivers, lakes and polar waters and they exhibit a diverse range of cellular, morphological, structural and biochemical composition (Chu *et al*., 2004). Many species of freshwater microalgae contain useful chemical compounds and valuable products including high quality proteins, pigments (carotenoids, chlorophyll and phycobiliproteins), lipoprotein, lutein and bioactive compounds that are useful pharmaceutically, as well as for various industrial applications (Chu *et al*., 2004;

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Amaro *et al*., 2011; Wan-Loy, 2012). Among these microalgae is *Scenedesmus*, which belongs to the order Sphaeropleales of the family Scenedesmaceae that is frequently dominant in freshwater lakes and rivers (Borowitzka & Borowitzka, 1988; Guiry, 2014). Many species of this genus are being used worldwide for various purposes due to their ability to adapt to harsh environmental conditions, ability to grow rapidly and ease of cultivation and handling (Lürling, 2003; Pultz & Gross, 2004). Likewise *Scenedesmus* spp. has been used in many biotechnological applications due to its high nutritional content and bioactivities (Chacón-Lee & Gonzalez-Marino, 2010; Guedes *et al*., 2011a). This review aims to collate information from very limited literature available on the great potential of *Scenedesmus* spp. such as antibacterial activities, biotechnological applications and other bioactive compounds it possesses. In doing so, it will reveal the need for further studies to be carried out.

Bioactive Compounds in Scenedesmus *spp.*

Similar to other microalgae, *Scenedesmus* spp. is a rich source of bioactive compounds that are being utilised for the benefit of humanity. They have been exploited for their active metabolites that have been applied in various industries including pharmaceutical, food, cosmetics, energy, aquaculture, medicine and others (Table 1). Some of the bioactive compounds are briefly discussed below.

Astaxanthin

It is considered to be one of the best carotenoid compounds that can successfully protect cells, lipids and membrane lipoproteins from oxidative damage (Ranga Rao *et al*., 2014). It is a sought-after compound as it is used in food, cosmetics and pharmaceutical applications (Kim *et al*., 2011). Astaxanthin products are commonly found in the form of soft gel, capsules, powder, tablet, oil, energy drinks and creams but they are mostly found in combination with other herbal extracts from other sources (Ranga Rao *et al*., 2014). Its other biological functions include; enhances immune response, acts as a strong coloring agent, protects against UV light and is a strong potent antioxidant (Guerin *et al*., 2003). Other carotenoids of importance include β-carotene and lutein, whose most important applications are as natural food colorants and in animal feed (Vilchez *et al*., 1997; Del Campo *et al*., 2000).

Vitamins

Microalgae are known to be a non-conventional source of vitamins because they possess several lipid-soluble and water vitamins in much higher concentrations over known conventional food (Kay, 1991; Zhang & Lee, 1997). According to Abd El Baky and El Baroty (2013), the vitamin content of microalgae such as Vitamin C, B_1 and B_2 is significantly higher than that of higher order plants. The biological functions of Vitamin C includes; strengthens the immune system, traps free radicals, regenerates Vitamin E and activates intestinal absorption of iron (Burtin, 2003). B vitamins (B_1, B_2, B_{12})

TABLE 1
Some valuable metabolites found in *Scenedesmus* spp.

Metabolites	Applications	Study
Vitamin B	Health-Food	Becker (2004); Borowitzka (1988).
Vitamin C	Health-Food additives, Pharmaceutical	Becker (2004); Borowitzka 1988.
Vitamin E	Health-Food, Medicine	Becker (2004); Borowitzka (1988).
Lutein	Animal nutrition, Pharmaceuticals	Tukaj *et al*. (2003); Otto & Wolfgang (2004); Ceron *et al*. (2008); Skjanes *et al*. (2013).
Astaxanthin	Aquaculture, cosmetics, Human nutrition, Medicine	Otto & Wolfgang (2004); Qin *et al*. (2008); Gouveia *et al*. (2008), Jouni and Makhoul, (2012).
Haemagglutinin	Medicine	Chu *et al*. (2004).
β- Carotene	Food colourant, Medicine	Karen *et al*. (2000); Indira & Biswajit (2012); Guedes *et al*. (2013).
Mycosporine-like amino acids, sporopollenin	Cosmetics (UV – screening compounds)	Indira & Biswajit, 2012; Skjanes *et al*. (2013).
Chlorophyll a, b, c	Food colourants, Pharmaceuticals, cosmetics	Karen *et al*. (2000); Gouveia *et al*. (2008); Indira & Biswajit, 2012; Catarina *et al*. (2013).
Polysaccharides	Medicine, Bioethanol, $BioH_2$	Gouveia *et al*. (2008); Skjanes *et al*. (2013).
Extracts with antimicrobial/ antifungal activities	Medicine	Abedin & Taha (2008).
Monounsaturated, polyunsaturated and Saturated fatty acids (Oleic acid, lauric acid, palmitic acid, linoleic acid, α-linoleic acid, stearic acid and others)	Biodiesel, Pharmaceuticals, Animal and Human nutrition, Aquaculture.	Ahlgren *et al*. (1992); Becker (2004); Kim *et al*. (2007); Gouveia and Oliveira (2009); Pandian and David (2012); Mahale and Chaugule (2013).
Amino acids (isoleucine, leucine, valine, lysine, methionine, cysteine, alanine, arginine, aspartic acid, glutamine and others)	Food	Chacón-Lee & Gonzalez-Marino (2010).

are used in the treatment of anemia, and has effect on ageing and chronic fatigue syndrome (Herrero *et al*., 2013).

Polysaccharides

Studies carried out with *Scenedesmus* spp. and other microalgae have shown that certain polysaccharides have medical effects (Skjanes *et al*., 2013). These polysaccharides function as protection against oxidative stress and have efficacy on gastric ulcers, wounds and constipation (Iwamoto, 2004; Spolaore *et al*., 2006).

Mycosporine-like amino acids

These are a group of molecules that consist of an amino acid that is bound to a chromophore and absorbs low wavelength light. These molecules play a vital role in protecting the microalgae against UV radiation (Skjanes *et al*., 2013). For this characteristic, they are being exploited for commercial purposes in cosmetic skin-care products for UV protection (Schmid *et al*., 2006).

Extraction of Biologically Active Compounds from Scenedesmus *spp.*

This is an important aspect to be considered in order to extract and isolate compounds of interest effectively. The general techniques of plant and algae extraction include maceration, hot continuous extraction (soxhlet), microwave-assisted extraction, sonication, supercritical fluid extraction, ultrasound assisted extraction, pressurised liquid extraction and hand grinding with pestle and mortar (Herrero *et al*., 2013). All of these have been also employed in the extraction of bioactive compounds from *Scenedesmus* algae. According to Herrero *et al*., (2013), successful determination of biologically active compounds from plants is also largely dependent on the type of solvent used in the extraction process. The choice of solvent used is influenced by what the extract is intended for and the targeted compounds.

In extracting different bioactive compounds from *Scenedesmus* spp. one of the techniques used is solvent extraction. Among important bioactive compounds extracted by solvent extraction methods from *Scenedesmus* include pigments, fatty acids, antioxidants and others (Table 2).

Antibacterial Activities of *Scenedesmus* spp.

Scenedesmus spp. has been reported to produce antimicrobial substances, which from the pharmaceutical's point of view, are a good source of new bioactive compounds. Although the potential of fatty acids to inhibit the growth and survival of pathogenic bacteria has been recognised for several years, the specific mechanism underlying the bactericidal action of fatty acids in microalgae remains to be fully understood. Nevertheless, they apparently enhance membrane damage that eventually enables cell leakage. Recently, studies of its structure-function relationship make it more evident that these antimicrobial activities rely on both the chain length and the degree of unsaturation (Guedes *et al*., 2011b).

TABLE 2
Different solvent extraction methods used for *Scenedesmus* spp.

Solvent	Extraction method	Compound extracted/ Activity	Study
100% Nanograde Acetone, 2:1 Dichloromethane/Methanol Quartz sand	Ultrasound, Sonication, Mechanical and hand grinding	Pigments and fatty acids	Karen *et al.* (2000).
Acetone, Ethanol Methanol, Diethyl ether	-	Antibacterial and antifungal activity	Abedin & Taha (2008).
1:1 Ethanol/Water	Homogenisation	Antibacterial activity	Catarina Guedes *et al.* (2011).
1:10 Ethanol/Acetone	-	Haemagglutination assay	Chu *et al.* (2004).
1:2 Chloroform/Methanol	Homogenisation	Lipid	Rajiv (2011).
3/1 v/v Ethanol/water Hexane, Ethyl acetate and Hot Water	Grounded with pestle and mortar	Antioxidants (phenols and carotenoids)	Koen *et al.* (2012),
90% Acetone	Hand shaking	Pigments	Beena & Krishnika (2011).
25ml of Diethyl ether, 25ml of petroleum ether.	Hand shaking	Fatty acid	Mahale & Chaugule (2013).
Acetone, Methanol, Dichloro Methane, Diethyl ether, Hexane, Hot Water, Cold water.	-	Antibacterial activity	Beena & Krishnika (2011).
Distilled water 100% ethanol	Ultrasonication	Antimicrobial and anticancer activity	Ördög *et al.* (2004).

Desbois *et al*. (2009) claimed that *Scenedesmus costatum* exhibited antibacterial activity against aquaculture bacteria as a result of their more than 10 carbon atoms in chain length of fatty acids. The Chlorophyta, or green algae, which include *Scenedesmus*, have chlorophyll a, b and several carotenoids (Tomaselli, 2004). Chlorophyll is one of the most valuable bioactive compounds that are being extracted from microalgal biomass and has gained importance as a food additive (Humphrey, 2004). Chlorophylls and β-carotene are major pigments present in microalgae that are known to act effectively as microbial growth inhibitors, and studies have shown that it has antioxidant as well as antimicrobial properties (Humphrey, 2004; Bhagavathy *et al*., 2011). Fan *et al*. (2013) and Jaya *et al*. (2007) reported that pigments from microalgae had antibacterial effect on certain bacteria. Patented application of astaxanthin is also available for preventing bacterial infections (Jouni & Makhoul, 2012). Furthermore, Guedes *et al*. (2011a) reported that *Scenedesmus* spp. is among

the few members of the green algae to produce antimicrobial substances and had active and prominent antibacterial properties that inhibited the growth of several pathogenic strains of bacteria when tested against them. These include *Salmonella* sp., *Pseudomonas aeroginosa, Escherichia coli* and *Staphylococcus aureus.*

Table 3 clearly shows that the antibacterial activity of *Scenedesmus* spp. extracts has positive results but with varying degrees of significant zones of inhibition. It also shows that *Scenedesmus* spp. extracts have higher antibacterial activity against the Gram positive bacteria than the Gram negative bacteria. According to Ördög *et al*. (2004), antibiotics are usually less effective against Gram- negative bacteria because of their more complex multilayered cell wall structure in addition to the presence of lipopolysaccharides on the outer cell wall thereby preventing the penetration of active compounds.

From the table, it is clear that various studies and screenings have been undertaken on this microalga pointing to its potential pharmaceutically. But there is yet to be a conclusive identification and characterisation of specific metabolites responsible for antibacterial activities.

Biotechnological Applications of *Scenedesmus* spp.

Today, industrial and commercial use of microalgal biomass and extracts of biomass has gained a strong foothold in various sectors of human life due to the presence of different useful compounds in them. The rich metabolic content of *Scenedesmus* spp. is being exploited for use in food (Becker, 2004; Toyub *et al*., 2008), aquaculture (Toyub *et al*., 2008), bioremediation (Martinez *et al*., 2000; Omar, 2002), cosmetics (Indira & Biswajit, 2012), pharmaceutical industries (Becker, 2004) and others. Some of these applications are briefly discussed below.

Nutraceutical Applications

Nutritional composition of *Scenedesmus* spp

Similar to higher order plants, the chemical composition of algae is not constant as it is determined by factors like environmental, temperature, pH value, mineral contents, CO_2 supply, population, density, growth phase and algae physiology that can modify its chemical composition (Gouveia *et al*., 2008). According to Yamaguchi (1997), microalgae have the ability to biosynthesise, metabolise, store and also secrete a diverse range of primary and secondary metabolites. Microalgal biomass is made up of different nutritional components of which the main three are proteins, carbohydrates and lipids (oil) (Indira & Biswajit, 2012). The following are some of the reasons why microalgae came to be of such commercial importance due to its nutritional composition: (1) the presence of high protein content in microalgae is the main reason it should be considered as a conventional source of protein, (2) its amino acid pattern compares favourably with other foods, (3) carbohydrates are obtained in various forms such as starch, glucose,

TABLE 3
Antibacterial inhibition of various *Scenedesmus* spp.

Staphylococcus aureus	*Streptococcus Pyogenes*	*Escherichia coli*	*Salmonella typhi*	*Bacillus subtilis*	*Enterococcus faecalis*	Zone of inhibition / Minimum inhibitory concentration	References
8	N/D	N/D	9	2	N/D	mm	Ghasemi *et al.* (2007)
16	16	N/D	N/D	N/D	N/D	mm	Najdenski *et al.* (2013)
18	18	N/D	N/D	N/D	N/D	mm	Najdenski *et al.* (2013)
17	17	N/D	N/D	N/D	N/D	mm	Najdenski *et al.* (2013)
1.0	N/D	R	R	4.0	N/D	cm	Abedin & Taha (2008)
1.5	N/D	R	R	3.0	N/D	cm	Abedin & Taha (2008)
R	N/D	R	R	2.5	N/D	cm	Abedin & Taha (2008)
1.2	N/D	1.5	R	2.0	N/D	cm	Abedin & Taha (2008)
5.0	N/D	N/D	N/D	N/D	N/D	mm	Ördög *et al.* (2004)
0.028	N/D	0.056	N/D	N/D	0.056	mg/ml	Ördög *et al.* (2004)

Note: R- Resistant, N/D- Not done

sugars, other polysaccharides and, (4) its total digestibility is extremely high, which explains why there are no limitations to its use in food and feed (Cornet, 1998; Becker, 2004; Solletto *et al*., 2005).

Scenedesmus spp. are particularly found to contain all essential amino acids and a good amount of protein, lipid and essential minerals (Geldenhuys *et al*., 1988). According to Becker (2004) and Batista *et al*. (2007), *Scenedesmus* contains lipids, proteins and carbohydrates that can compare favourably with other food protein (Table 4).

Human Nutrition

Microalgae for human nutritional requirements are currently being manufactured in different forms such as tablets, capsules, pastilles, liquids and nutritional supplements and are also incorporated into snacks, pastas, candy bars or chewing gum and in beverages (Spolaore *et al*., 2006; Gouveia *et al*., 2008). *Scenedesmus* is among the most used microalgae that has attracted the attention of manufacturers in the food and health-food market (Chacón-Lee & Gonzalez Marino, 2010). Compared to case in, *Scenedesmus* has very high nutritional quality and several toxicological assessments have not revealed any toxic impacts or abnormalities in experiments with test animals (Becker, 1984). Gross *et al*. (1978) carried out a nutritional study by incorporating *Scenedesmus* sp. into the diet of children (5 g/daily) and adults (10 g/daily) and a slight increase in weight was discovered. Subsequently, there was a significant improvement in the weight of four-year-old children who were fed with microalgae compared to those fed with a normal diet. In another study carried out by Natrah *et al*. (2007), a *Scenedesmus* sp. among other microalgae was shown to possess antioxidant properties and biochemical contents that could be applied in the nutraceutical industry.

TABLE 4
Nutrient composition of different *Scenedesmus* spp. (% dry matter)

Scenedesmus spp./Food products	Protein	Carbohydrate	Lipid	Ash	Crude	References
S. obliquus	50-56	10-17	12 -14	N/A	N/A	Becker (2004)
S. dimorphus	8-18	21-52	16 -40	N/A	N/A	Um & Kim (2009); Sydney *et al*. (2010)
S. acutus	50-60	10-17	12 - 14	6 – 10	3 - 10	Soeder & Prabst (1970)
S. quadricauda	47	N/A	1.9	N/A	N/A	Um & Kim (2009); Sydney *et al*. (2010).
S. obliquus	6-12	33-64	11 - 21	N/A	N/A	Batista *et al*. (2007)
S. dimorphus	60-70	13-16	6 - 7	N/A	N/A	Batista *et al*. (2007)
S. obliquus	34.5	N/A	16.13	12.0	6.39	Toyub *et al*. (2008)

N/A- Not applicable

Potential Source of Biodiesel

Due to global warming and exhaustion of fossil fuels, which has become a worldwide problem due to the emission of greenhouse gasses (GHG), attempts have been made to find alternative sources of energy from various biological materials such as plants, animal fat and microalgae (Pandian & David, 2012). They showed in a study that *Scenedesmus* spp. had a high oleic acid content of about 52.8%, making it most suitable for the production of good quality biodiesel.

Bio hydrogen ($BioH_2$) production

Scenedesmus sp. has been used as a feedstock for $BioH_2$ production as a source of biofuel to power both light and heavy-duty vehicles, as well as jet and marine engines (Gouveia *et al*., 2012). Currently, all major car producers offer cars running on hydrogen as fuel; it was discovered that *Scenedesmus* spp. was able to produce hydrogen, and this discovery has led to the search for a way to use this microalgae to convert solar energy into this useful energy carrier (Skjanes *et al*., 2013).

Wastewater treatment

Treatment of waste is an important problem in the world due to the increase in population and industrial activities. Agricultural and municipal wastes contain all the macro and micro-nutrients that are needed for algal growth; therefore, it is economical to grow algae in wastewater (Toyub *et al*., 2008). Because of the special ability of *Scenedesmus* spp. to adapt to different environmental conditions it is being used in domestic and industrial wastewater treatment. It has recently been used in removing heavy metals and in the production of oxygen and in converting waste products into beneficial substances (Abuzer *et al*., 2008).

Cosmetic industry

Several compounds of *Scenedesmus* spp. are used in cosmetic industry as thickening agents, water-binding agents and antioxidants in facial and skin care products (Rajiv, 2011). Pigments such as carotenoids i.e. astaxanthin, phycocyanine and β-carotene (Sánchez *et al*., 2007), mycosporine-like amino acids and sporopollenin are extracted and used as UV screening compounds for skin protection in cosmetics (Indira & Biswajit, 2012: Skjanes *et al*., 2013). Amino acids are known to have a universal function in proteins, but they are also important for skin hydration, elasticity and photoprotection and are included in cosmetics (Lebeau & Robert, 2003).

Pharmaceutical industry

Microalgae have for a long time been used for their therapeutic powers, but scientific investigations for biologically functional compounds started in the 1950s and since then, extensive research has been conducted to find compounds that might result in therapeutically beneficial agents (Mendes *et al*., 2003; Mayer & Hamann, 2005; Cardozo *et al*., 2007; Amaro *et al*., 2011). Furthermore, microalgae has

been found to produce antibiotics as its extracts have proven to be antibacterial, anti-protozoal and antiplasmodial, and it is mostly accredited to compounds belonging to the following chemical classes: indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons (Kellan & Walker, 1989; Ghasemi *et al*., 2004; Ozemir *et al*., 2004; Herrero *et al*., 2006; Cardozo *et al*., 2007).

The three major classes of microalgal photosynthetic pigments (chlorophyll, carotenoids and phycobilins) have demonstrated biological activity in a wide range of biological applications, including prevention of acute and chronic coronary syndromes, muscular dystrophy, atherosclerosis, cataract, rheumatoid arthritis and neurological disorders (Schoefs, 2004; Mimouni *et al*., 2012).

Scenedesmus produces polysaccharides that have been shown to have medical effects that act against oxidative stress (Mohammed, 2008). Other compounds found in *Scenedesmus* that have therapeutic abilities include Vitamin E (Pham-Huy *et al*., 2008), Vitamin C (Skjanes *et al*., 2013), astaxanthin (Olaizola, 2003) and metabolites with antibiotic activities (Chu *et al*., 2004; Ördög *et al*., 2004).

CONCLUSION

Scenedesmus spp. as a rich source of bioactive metabolites presents an advantage and an opportunity for its use in various applications that include aquaculture, cosmetics, pharmaceuticals and human nutrition. Studies had suggested that *Scenedesmus* spp. can produce diverse chemical compounds especially long-chain polyunsaturated fatty acids that can inhibit the growth of pathogenic micro-organisms. However, the active compounds in *Scenedesmus* have not being applied as antibacterial agents to inhibit food-borne pathogens such as *Staphylococcus aureus*, *Bacillus cereus* and *Salmonella* spp. in food preparation and production. Therefore, further studies particularly in the application of the potential of antibacterial compounds in food industry have to be carried out.

REFERENCES

Abd El Baky, H. H., & El Baroty, G. S. (2013). Healthy benefit of microalgal bioactive substances. *Journal of Aquatic Science*, *1*(1), 11-23.

Abedin, R. M. A., & Taha, H. M. (2008). Antibacterial and antifungal activity of cyanobacteria and green microalgae. Evaluation of medium components by Plackett-Burman design for antimicrobial activity of *Spirulina Platensis. Global Journal of Biotechnology and Biochemistry, 3*(1), 22-31.

Abuzer C., Muharrem B., & Husseyin B. (2008). Modeling of *Scenedesmus Obliquus*; function of nutrients with modified Gompertz model. *Bioresource, 99*(18)*,* 8742-8747.

Ahlgren, G., Gustafsson, I. B., & Boberg, M. (1992). Fatty acid content and chemical composition of fresh water microalgae. *Journal of Phycology, 28*(1), 37-50.

Amaro, H. M, Guedes, A. C, & Malcata, F. X. (2011). Antimicrobial activities of microalgae: An invited review. In A. *Méndez-Vilas (Ed.)*, *Science against microbial pathogens: Communicating current research and technological advances* (pp. 1272-1284). Formatex Microbiology Book Series, Nova Science Publisher.

Batista, A. P., Bandarra, N., Raymundo, A., & Gouveia, L. (2007). Microalgae biomass-a potential ingredient for the food industry. *EFFoST/EHED Joint Conference*. Lisbon, Portugal.

Becker, E. W. (1984). Biotechnology and exploitation of the green alga *Scenedesmus obliquus* in India. *Biomass*, *4*(1), 1-19.

Becker, W. (2004). Microalgae in human and animal nutrition. In A. Richmond (Ed.), *Handbook of microalgal culture: Biotechnology and applied phycology* (pp. 312-351). Oxford: Blackwell Science Ltd.

Beena, B. N., & Krishnika, A. (2011). Antibacterial activity of freshwater microalga (*Scenedesmus* sp.) against three bacterial strains. *Journal of Bioscience Resources, 2*(4), 160-165.

Bhagavathy, S., Sumathi, P., & Jancy, S. B. I. (2011). Green algae *Chlorococcum humicola* - A new source of bioactive compounds with antimicrobial activity. *Asian Journal of Tropical Biomedicine*, *1*(1) S1-S7.

Borowitzka, M. A. (1988). Vitamins and fine chemicals from micro-algae. In M. A. Borowitzka, & L. J. Borowitzka (Eds.), *Micro-algal biotechnology* (pp. 153-196). Cambridge University Press, Cambridge.

Borowitzka, M. A., & Borowitzka, L. J. (1988). *Dunaliella*. In Borowitzka M. A., & Borowitzka L. J. (Eds.). *Micro-algal biotechnology* (pp. 27-58). Cambridge University Press: Cambridge.

Burtin, P. (2003). Nutritional value of seaweeds. *Electronic Journal of Environmental Agricultural Food Chemistry, 2*, 498-503.

Cardozo, K. H. M., Guaratini, T., Barros, M. P., Falcáo, V. R., Tonon, A. P., Lopes, N. P., … & Pinto, E. (2007). Metabolites from algae with economical impact. *Comparative Biochemistry and Physiology*, *146*(1), 60-78.

Catarina Guedes, A., Barbosa, C. R., Amaro, H. M., Pereira, C. I. & Xavier Malcata, F. (2011). Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. *International Journal of Food Science & Technology, 46*(4), 862-870.

Catarina, G. A., Maria, S. G., Ana, A. M., Ana, V. M. N., Manuela, E. P., Catarina, M. M. D., & Xavier M. F. (2013). Supercritical fluid extraction of carotenoids and chlorophylls a, b, c, from a wild strain of *Scenedesmus obliquus* for use in food processing. *Journal of Food Engineering, 116*(2), 478-482.

Ceron, M. C., Campos, I., Sanchez, J. F., Acien, F. G., Molina, E., & Fernandez-Sevilla, J. M. (2008). Recovery of lutein from Microalgae biomass: Development of a process for *Scenedesmus almeriensis* biomass. *Journal of Agriculture and Food Chemistry, 56*(24), 11761-11766.

Chacón-Lee, T. L., & González-Marino, G. E. (2010). Microalgae for healthy foods – Possibilities and challenges. *Comprehensive reviews in food science and food safety, 9*(6), 655-675.

Chu, C. Y., Liao, W. R., Huang, R., & Lin, L. P. (2004). Haemagglutinating and antibiotic activities of freshwater microalgae. *World Journal of Microbiology & Biotechnology, 20*(8), 817-825.

Cornet, J. F. (1998). Le technoscope: Les photobioréacteurs. *Biofutur, 176*(1), 1-10.

Del Campo, J. A., Moreno, J., Rodriguez, H., Vargas, M. A., Rivas, J., & Guererro, M. G. (2000). Carotenoid content of chlorophycean microalgae: Factors determining lutein accumulation in *Muriellopsis* sp. (Chlorophyta). *Journal of Biotechnology, 76*(1), 51-59.

Desbois, A. P, Mearns-Spragg, A., & Smith, V. J. (2009). A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial agent diverse bacteria including multi resistant *Staphylococcus aureus* (MRSA). *Marine Biotechnology*, *11*(1), 45-52.

Fan, M., Liao, Z., Wang, R., & Xu, N. (2013). Isolation and antibacterial activity of anabaena phycocyanin. *African Journal of Biotechnology, 12,* 1869-1873.

Geldenhuys, D. J., Walmsley, D. J., & Tofrien, D. J. (1988). Quality of algal material produced on a fertilizer-tap water medium in outdoor plastic enclosed systems. *Aquaculture, 68*(2)*,* 157-164.

Ghasemi, Y., Yazdi, M. T., Shafiee, A., Amini, M., Shokravi, Sh., & Zarrini, G. (2004). Parsiguine, a novel antimicrobial substance from *Fischerella ambigua. Journal of Pharmaceutical Biology*, *42*(4-5), 318-322.

Ghasemi, Y., Moradian, A., Mohagheghzadeh, A., Shokravi, S., & Morowvat, M. H. (2007). Antifungal and antibacterial activity of the microalgae collected from paddy fields of Iran: Characterization of antimicrobial activity of *Chroococcus dispersus*. *Journal of Biological Sciences, 7*(6)*,* 904-910.

Gouveia, L., Batista, A. P., Sousa, I., Raymundo, A., & Bandarra, N. M. (2008). Microalgae in novel food products In K. Papadoupoulos, *Food chemistry research developments*. Nova Science Publishers.

Gouveia, L., Batista, A. P., Nobre, B. P., Marques, P. A., Moura, P., Alves, L. M., ... & Palavra, A. F. (2012). *Exploring Scenedesmus obliquus and Nannochloropsis sp. Potential as a sustainable raw material for biofuels and high added value compounds.* Paper presented at the 1er. Congresso Iberoamericano Sobre Biorrefinerias, 1-CIAB, Los Cabos, Baja California, México, 24-26 October, 2012.

Gouveia, L., & Oliveira, A. C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology Biotechnology, 36*(2)*,* 269-274.

Gross, R., Gross, U., Ramirez, A., Cuadra, K., Collazos, C., & Feldheim, W. (1978). Nutritional tests with green Scenedesmus with health and malnourished children. *Archiv fur Hydrobiologie, Beihefte Ergebnisse der Limnologie*, *11*,174-183.

Guedes, A. C., Gião, M. S., Matias, A. A., Nunes, A. V. M., Duarte, C. M. M., Pintado, M. E., & Malcata, F. X. (2013). Supercritical fluid extraction of carotenoids – including lutein and chlorophylls a, b and c from a wild strain of *Scenedesmus obliquus* for use in food processing. *Journal of Food Engineering, 116*(2), 478-482.

Guedes, A. C., Catarina, R. B., Helena, M. A., Claudia, I. P., & Francisco, X. M. (2011a). Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. *International Journal of Food Science and Technology, 46*(4), 862-870.

Guedes, A. C., Amaro, H. M., & Malcata, F. X. (2011b). Microalgae as sources of high added-value compounds—a brief review of recent work. *Biotechnology Progress, 27*(3)*,* 597-613.

Guerin, M., Huntley, M. E., & Olaizola, M. (2003). *Haematococcus astaxanthin*: Applications for human health and nutrition. *Trends in Biotechnology, 21*(5)*,* 210-216.

Guiry, M. D. (2014). *Scenedesmus meyen*, 1829. In Guiry, & Guiry, (Eds.), *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway (taxonomic information republished from AlgaeBase with permission of M.D. Guiry). Retrieved on 2014 Aug, 5.

Herrero, M., Ibanez, E., Cifuentes, A., Reglero, G., & Santoyo, S. (2006). *Dunaliella salina* microalga pressurized liquid extracts as potential antimicrobials. *Journal of Food Protection*, *69*(10)*,* 2471-2477.

Herrero, M., Jose, M. A., Merichel, P., & Elena, I. (2013). Screening for bioactive compounds from alga. In James Weifu Lee (Ed.), *Advanced biofuels and bioproducts*. Springer New York Heidelberg Dordrecht London. DOI: 10.1007/978-1-46143348-4_35, 833.

Humphrey, A. M. (2004). Chlorophyll as a color and functional ingredient. *Journal of Food Science*, *69*(5), 422-425.

Indira, P., & Biswajit, R. (2012). Commercial and industrial applications of micro-algae – A review. *Journal of Algal Biomass Utln, 3*(4), 89-100.

Iwamoto, H. (2004). Industrial production of microalgal cell mass and secondary products - major industrial species – *Chlorella* ( ). In: A. Richmond (Ed.), *Handbook of microalgal culture*. Blackwell Oxford.

Jaya, P. G. M., Seshikala, D., & Singara, C. M. A., (2007). Antibacterial activity and biomolecular composition of certain freshwater micro-algae from River Godavari (India). *Science World Journal, 2*(3), 19-23.

Jouni, Z., & Makhoul, Z. (2012). *Patent US 20120238522, 2012, Sep 20*. Carotenoid containing compositions and methods.

Karen, H. W., Maarten, B., Anita, M., & Heinke, B. (2000). Extraction of pigments and fatty acids from the green alga *Scenedesmus Obliquus* (Chlorophyceae). *Aquatic Ecology, 34*(2)*,* 119-126.

Kay, A. R. (1991). Microalgae as food and supplement. Critical Reviews. *Food Science Nutrition*, *30*(6), 555-573.

Kellan, S. J., & Walker, J. M. (1989). Antibacterial activity from marine microalgae in laboratory culture. *British Phycological Journal, 24*(2), 191-194.

Kim, J. H., Chang, M. J., Choi, H. D., Youn, Y. K., Kim, J. T., Oh, J. M., & Shin, W. G. (2011). Protective effects of *Haematococcus astaxanthin* on oxidative stress in healthy smokers. *Journal of Medicinal Food*, *14*(11), 1469-1475.

Kim, M. K., Park, J. W., Park, C. S., Kim, S. J., Jeune, K. H., Chang, M. U., & Acreman, J. (2007). Enhanced production of *Scenedesmus* spp. (green microalgae) using a new medium containing fermented swine wastewater. *Bioresource Technology*, *98*(11)*,* 2220-2228.

Koen, G., Koenraad, M., & Ilse, F. (2012). Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of Applied Phycology, 24*(6), 1477-1486.

Lebau, T., & Robert, J-M. (2003). Diatom cultivation and biotechnological relevant products. Part II: Current and putative products. *Applied Microbiology Biotechnology*, *60*(6), 624-632.

Lürling, M. (2003). Phenotypic plasticity in the green algae *Desmodesmus* and *Scenedesmus* with special reference to the induction of defensive morphology. *Annales de Limnologie - International Journal of Limnology*, *39*(2), 85-101.

Martínez, M. E., Sánchez, S., Jiménez, J. M., El Yosufi, F., & Muñoz, L. (2000). Nitrogen and phosphorous removal from urban wastewater by the microalga *Scenedesmus obliquus*. *Bioresource Technology, 73*(3)*,* 263-272.

Mahale, V. E., & Chaugule, B. B. (2013). Optimization of freshwater green alga *Scenedesmus incrassatulus* for biomass production and augmentation of fatty acids under abiotic stress conditions. *Phykos, 43*(1), 22-31.

Mayer, M. N. S., & Hamann, M. T. (2005). Marine pharmacology in 2001-2002: Marine compounds with anthelmintic, antibacterial, anticoagulant, antidiabetic, antifungal, anti-inflammatory, antimalarial, antiplatelet, antiprotozoal, antituberculosis, and antiviral activities; affecting the cardiovascular, immune and nervous systems and other miscellaneous mechanisms of action. *Comparative Biochemistry and Phycology, 140*(3)*,* 265-286.

Mendes, R. L., Nobre, B. P., Cardoso, M. T., Pereira, A. P., & Palabra, A. F. (2003). Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorganica Chimica Acta*, *356*, 328-334.

Mimouni, V., Ulmann, L., Pasquet, V., Mathieu, M., Picot, L., Bougaran, G.,…& Schoefs, B. (2012). The potential of microalgae for the production of bioactive molecules of pharmaceutical interest. *Current Pharmaceutical Biotechnology*, *13*(15), 2733-2750.

Mohammed, Z. A. (2008). Polysaccharides as a response against microcystin-induced oxidative stress in *Chlorella vulgaris* and *Scenedesmus quadricauda* and their possible significance in the aquatic ecosystem. *Ecotoxicology*, *17*(6), 504-516.

Najdenski, H. M., Gigova, L. G., Iliev, I. I., Pilarski, P. S., Lukavský, J., Tsvetkova, I. V., Ninova, M. S., & Kussovski, V. K. (2013). Antibacterial and antifungal activities of selected microalgae and cyanobacteria. *International Journal of Food Science & Technology*, *48*(7), *1533*-1540.

Natrah, F. M. I., Yusoff, F. M., Shariff, M., Abas, F., & Mariana, N. S. (2007). Screening of Malaysian indigenous microalgae for antioxidant properties and nutritional value. *Journal of Applied Phycology*, *19*(6), 711-718.

Olaizola, M. (2003). Commercial development of microalgal biotechnology: from the test tube to the market place. *Biomolecular Engineering*, *20*(4), 459-466.

Omar, H. H. (2002). Bioremoval of zinc ions by *Scenedesmus obliquus* and *Scenedesmus quadricauda* and its effect on growth and metabolism. *International Biodeterioration Biodegradation*, *50*(2), 95-100.

Ördög, V., Stirk, W. A., Lenobel, R., Bancirova, M., Strnad, M., Van, S. J., Szigeti, J., & Nemeth, L. (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, *16*(4), 309-314.

Ozemir, G., Karabay, N.U., Dalay M.C., & Pazarbasi, B. (2004). Antibacterial activity of volatile components and various extracts of *Spirulina platensis*. *Phytotherapy Research*, *18*(9), 754-757.

Pandian, P., & David, R. A. (2012). *Scenedesmus* as a potential source of biodiesel among selected microalgae. *Current Science, 102*(4), 616-620.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science, 4*(2), 89-96.

Pultz, O., & Gross, W. (2004). Valuable products from biotechnology of microalgae. *Applied Microbiology Biotechnology, 65*(6), 635-648.

Qin, S., Liu, G. X., & Hu, Z. Y. (2008). The accumulation and metabolism of astaxanthin in *Scenedesmus obliquus* (Chlorophyceae). *Process Biochemistry, 43*(8), 795-802.

Rajiv, C. D. G. (2011). *Scenedesmus dimorphus* and *Scenedesmus quadricauda*: Two potent indigenous microalgae strains for biomass production and Co_2 mitigation –A study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source. *Journal of Algal Biomass Utilization*, *2*(4), 42-49.

Ranga Rao, A., Siew Moi, P., Sarada, R., & Ravishankar, G. A. (2014). Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—A review. *Marine Drugs, 12*(1), 128-152.

Sánchez, J. F., Fernández, J. M., Acién, F. G., Cerón, M. C., Pérez-J., & Molina, E. (2007). Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis. Process Biotechnology Chemistry*, *43*(4), 398-405.

Schmid, D., Schürch, C., & Zülli, F. (2006). Mycosporine-like amino acids from red algae protect against premature skin-aging. *European Cosmetics, 9,* 1-4.

Schoefs, B. (2004). Determination of pigments in vegetables. *Journal of Chromatography*, *1054*(1)*,* 217-226.

Skjanes, K., Celine, R., & Peter, L. (2013). Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. *Critical reviews in Biotechnology, 33*(2), 172-215.

Soeder, C. J., & Pabst, W. (1970). Gesichtspunkte für die Verwendung von Mikroalgen in der Ernährung von Mensch und Tier1. *Berichte der Deutschen Botanischen Gesellschaft*, *83*(11)*,* 607-625.

Soeder, C. J., & Hegewald, E. (1992). *Scenedesmus*. In M. A. Borowitzka, & Borowitzka (Eds.). *Microalgal biotechnology.* Cambridge: Cambridge University Press.

Soletto, D., Binaghi, L., Lordi, A., Carvalho, J. C. M., & Converti, A. (2005). Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. *Aquaculture, 243*(1)*,* 217-224.

Spolaore, P., Claire, J. C., Elie, D., & Arsene, I. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering, 101*(2), 87-96.

Sydney, E. B., Sturm, W., de Carvalho, J. C., Thomaz-Soccol, V., Larroche, C., Pandey, A., & Soccol, C. R. (2010). Potential carbon dioxide fixation by industrially important microalgae. *Bioresource Technology*, *101*(15)*,* 5892-5896.

Tomaselli, L. (2004). The microalgal cell. In A. Richmond (Eds.), *Handbook of microalgal culture: biotechnology and applied psychology* (pp.3-19). Oxford: Blackwell Publishing Ltd.

Toyub, M. A., Miah, M. I., Habib, M. A. B., & Rahman, M. M. (2008). Growth performance and nutritional value of *Scenedesmus obliquus* cultured in different concentrations of sweetmeat factory waste media. *Bangladesh Journal of Animal Science, 37*(1), 86-93.

Tukaj, Z., Matusiak, M. K., Lewandowska, J., & Szurkowski, J. (2003). Changes in the pigment pattern and the photosynthetic activity during a light-induced cell circle of the green alga *Scenedesmus armatus*. *Plant Physiology Biochemistry, 41*(4)*,* 337-344.

Um, B. H., & Kim, Y. S. (2009). Review: A chance for Korea to advance algal-biodiesel technology. *Journal of Industrial and Engineering Chemistry, 15*(1), 1-7.

Vilchez, C., Garbayo, I., Lobato, M. V., & Vega, M. J. (1997). Microalgae-mediated chemicals production and wastes removal. *Enzyme Microbiology Technology*, *20*(8)*,* 562-572.

Wan-Loy, C. (2012). Biotechnological applications of microalgae. *IeJSME 2012, 6* (Suppl 1), S24-S37.

Yamaguchi, K. (1997). Recent advances in microalgal bioscience in Japan, with special reference to utilization of biomass and metabolites: a review. *Journal of Applied Phycology, 8*(6), 487-502.

Zhang, D. H., & Lee Y. K. (1997). Enhanced accumulation of secondary carotenoids in a mutant of green algae *Chlorococcum* sp. *Journal of Applied Phycology*, *9*(5)*,* 459-463.