REVIEW ARTICLE

Bioactive Compounds, Cosmeceutical And Nutraceutical Applications of Green Seaweed Species (*Chlorophyta*)

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Abstract

Seaweeds are valuable marine plants that have garnered much attention from the public due to their high bioactive, nutrients and minerals content. Seaweeds have been used in multiple applications, including in cosmeceutical, nutraceutical and pharmaceutical industries. Nevertheless, this review will focus on the bioactive compounds of Chlorophyta and their potential application in nutraceutical and cosmeceutical industries. Chlorophyta are believed to possess a significant amount of nutrients and minerals, sufficient to meet the daily requirements of nutrients and minerals in the human body. Considering the nutritional aspect, deficiency in nutrients may lead to severe ailments, including heart disease, neurological disorder and cancer. The main compounds studied in this review are polysaccharides, proteins, amino acids, lipids, fatty acids, pigments, minerals, vitamins and secondary metabolites. Among all, polysaccharides are the most exploited compounds and used in many advanced applications in the nutraceutical and cosmeceutical industries. This review also offers insights into the beneficial biological properties of Chlorophyta, highlighting their potential in cosmeceutical and nutraceutical applications. Further research is required to highlight the Chlorophyta sp. aquaculture, its extraction method, and the most targeted bioactive compounds from the species. Therefore, the challenge is to increase public awareness of the promising application of this species in the nutraceutical and cosmeceutical industries.

Keywords: chlorophyta, cosmeceutical, nutraceutical

Introduction

Seaweeds, also known as macroalgae, are composed of large, diverse macroscopic, eukaryotic, photosynthetic and non-vascular marine organisms. Garcia-Vaquero, Lopez-Alonso, and Hayes (2017) had listed 10,000 different species of seaweeds, which occupy the littoral zone. The structure of seaweeds is varied, as some are filamentous with a few millimeters in height, while others have huge fronds up to 60meter long. Also, the seaweeds' chemical structure and bioactive compounds are varied based on their habitats, which are either in the harsh marine or terrestrial environment (Garcia-Vaquero et al., 2017). Seaweeds are classified into three higher taxa, namely Chlorophyta (green seaweed), Phaeophyta (brown seaweed) and Rhodophyta (red seaweed), based on their pigmentation.

Seaweeds have been consumed as a type of sea vegetable in most countries such as Japan, China and Korea. They have become a source of hydrocolloids (alginate, carrageenan, and agar), thickening and gelling agents and had been utilized in industrial and foods across Western countries (Pereira, 2018). At present, there is a high demand for seaweeds as many quarters have begun consuming healthy and 'natural foodstuffs', mainly because seaweeds are rich in minerals, vitamins, and proteins. Additionally, the French consumed several types of macroalgae and microalgae in their meals as vegetables or condiments. They have also been widely known and used as a source of fertilizer and thickening agents. Meanwhile, in Japan, seaweeds make up to 10-25 % of food intake as they used seaweeds as sushi wrappings, seasonings, condiments and vegetables (Miyamoto, Yabuta, Kwak, Enomoto, & Watanabe, 2009). Seaweeds species have been in high demand in



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[®]Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 2021. Accreditation Number:148/M/KPT/2020. ISSN: 2089-5690, e-ISSN: 2406-9272. doi: 10.15578/squalen.514 most industries including nutraceutical and cosmeceutical as they contain high carbohydrates, proteins, fiber, vitamins, minerals and low-fat content. Also, seaweeds are an excellent source of B group vitamins (B1, B2, and B12), vitamins with antioxidant activities, vitamins C and E, provitamin A and carotenoids (Škrovánková, 2011). Hence, seaweeds can be an alternative food or supplement for those following a special diet and strict vegetarians. Yaich et al. (2011) asserted that several seaweed species, such as *Ulva* sp., have been authorized for human consumption. Therefore, it can be deduced that seaweeds have become valuable vegetables and an essential food ingredient in the human diet. Figure 1 shows seaweed (*U. lactuca*) in *Chlorophyta* family.

According to Pati, Sharma, Nayak, and Panda (2016), the bioactive component such as carbohydrate content in the Chlorophyta family is higher than that in Rhodophyta and Phaeophyta, which also depending on their species and habitats. For instance, higher carbohydrate content was recorded in U. lactuca (35.27 %) from Chlorophyta, compared to that in Dictvota dichtoma (10.63 %) from Phaeophyta family. Priyan, Kim, Kim, and Jeon (2019) also discussed that carbohydrates from Chlorophyta had been proven to exhibit many cosmeceutical properties such as antioxidant, anti-wrinkle and moisturizing properties. In a study conducted by Roleda et al. (2021), they discussed the Chlorophyta sp. for its nutritional content that will benefit the public, anchored on the presence of polysaccharides, pigments, fatty acids, polyphenols and peptides. These compounds may as well contribute to the development of nutraceutical and cosmeceutical. Several studies highlighted the utilization of Monostroma latissimun, C. racemosa, U. lactuca and, U. australis in nutraceutical applications. Most Asian countries such as China, Japan and Korea have consumed these seaweed species as medicinal foods,

dietary supplements and fortified products for human consumption through their diets. This is due to the biologically active compounds present in these species, such as carbohydrates, dietary fibers, vitamins, minerals and others that provide great human health benefits and deputized for an inexhaustible source of materials for the nutraceutical and cosmeceutical applications (Cotas, Leandro, Pacheco, Gonçalves, & Pereira, 2020).

However, seaweeds from *Chlorophyta* sp. appear to be unexploited, especially when compared to Rhodophyta and Phaeophyta. This species has not been utilized optimally by the community due to limited research work concerning this species, despite their beneficial application for human diet. Therefore, the focus of this review is narrowed down to green seaweed, Chlorophyta sp., by emphasizing the bioactive compounds in this species that had been explored and utilized in the nutraceutical and cosmeceutical industries. Generally, a nutraceutical compound is defined as a compound that will intensify the food products' benefits when added. Meanwhile, cosmeceutical compounds will add a therapeutic value on cosmetic products (Cotas et al., 2020). A few typical examples of Chlorophyta sp. were successfully discovered for their bioactive compounds and applications, namely Ulva, Monostroma, Enteromorpha and Caulerpa (Kumar, Ganesan, Suresh, & Bhaskar, 2008).

Nevertheless, only a handful of studies have assessed biological and nutritional contents in *Chlorophyta* sp. This is because less research was conducted concerning the extraction of bioactive compounds from this species than those from other species. This review probed into these seaweed species to identify their nutritional values and their potential benefits in the nutraceutical and cosmeceutical applications.



Figure 1. Ulva lactuca, from Chlorophyta collected from Merambong Island, Johor Bahru, Malaysia.

Bioactive Compounds in Chlorophyta

In Malaysia, plenty of seaweed species can be found in Merambong Island, located in Johor state. Merambong Island offers a vast range of habitats, apart from providing a stable environment that is adequate to support the growth of these seaweeds (Zainee, Ismail, Taip, Ibrahim, & Ismail, 2019). According to Zainee et al. (2019), there are 25 genera, including 46 species of seaweeds found in Merambong Island. *Chlorophyta* was found to be the highest inhabitant in Merambong Island, accommodating 11 genera, followed by *Rhodophyta* (10 genera) and *Phaeophyta* (4 genera). Therefore, this review focused on the highest inhabitant in Merambong island, which is the green seaweed, *Chlorophyta* sp.

Chlorophyta sp. are richer in nutritional contents than plants that live on land because they utilize only a small amount of energy to form circulatory systems, leaves, roots, stem and reproductive organs. Thus, more phytonutrients, protein and lipids can be stored (Hasan, 2017). There are several bioactive and nutritional compounds reported in the *Chlorophyta* sp., e.g., natural pigments (NPs), polyunsaturated fatty acids (PUFAs), lipids, proteins and polysaccharides (Khalid, Abbas, Saeed, Bader-Ul-Ain, & Ansar Rasul Suleria, 2018; Kumar et al., 2008). These commercial bioactive compounds are believed to possess many potential health benefits that later may be utilized in nutraceutical and cosmeceutical industries. However, environmental changes such as changes in light, nutrients, contaminants, salinity, pH, temperature and CO_a availability may cause variation of bioactive compounds present in seaweeds (Khalid et al., 2018).

Polysaccharides

Marine plants such as seaweed contain many polysaccharides, which can be found in their cell wall structure (Kumar et al., 2008). Polysaccharides are complex biological macromolecules that constitute monosaccharides polymers connected by glycosidic (ether) links. The extracted polysaccharides are usually found in sulfated and non-sulfated forms (Hentati et al., 2020). Generally, the polysaccharides (matrix and storage) present in the three different seaweed species (Chlorophyta, Phaeophyta, and Rhodophyta) are macroalgae species-specific. For instance, ulvans and xylans were found abundantly in Chlorophyta (Hentati et al., 2020; Kumar et al., 2008). In general, ulvan is differentiated from other seaweed polysaccharides because of the remarkable rare sugars rhamnose and iduronic acid, which are identical to the mammalian glycosaminoglycans (Figueira, da Silva, Enrich-Prast, Yoneshigue-Valentin, & de Oliveira, 2020). Meanwhile, xylans have a molecular structure of $1,4-\beta$ -D-xylans like higher plants (Aizatul, Abdul, Rahim, Yusof, & Atikah, 2021).

Ulvans

The cell walls of *Chlorophyta* sp. are made up of ulvan. It is a sulfated polysaccharide that consists of a central framework of disaccharide modules, Lrhamnose 3-sulphate, which is linked to (i) ulvabiouronic acid unit A; (ii) ulvabiuronic acid unit B; (iii) ulvabiose unit A; or (iv) ulvabiose unit B (Shah et al., 2020). Moreover, ulvan is mainly composed of variable amounts of rhamnose, glucuronic acid, iduronic acid, xylose and high level of charged sulfated polyelectrolytes (Figueira et al, 2020; Aizatul et al., 2021). Ulva (family Ulvacea) from Chlorophyta sp. offered a wide range of nutraceutical and pharmacological applications since they have the capacity to manufacture ulvans moieties of different sugar units (Cunha & Grenha, 2016). For instance, a low molecular weight (28.2 kDa) ulvans of U. pertusa exhibited high inhibitory activity against hydroxyl and superoxide radicals, also showed a strong reduction capacity and metal chelating properties (Shah et al., 2020). Thus, these properties can be further exploited in the industries for their powerful antioxidant agents. In addition, studies also showed that ulvans from Chlorophyta sp. manifested the potentiality as an antiviral, anticancer and anti-aging (Figueira et al., 2020). Although many studies have discussed the exploitation of polysaccharides from other seaweeds species in the industries, ulvans from Chlorophyta sp. are largely untapped.

Xylans

Xylans can be found in the cell wall of *Chlorophyta* sp. such as in the *Caulerpa*, which is composed of 1,3- β -D-xylans (Hsieh & Harris, 2019). In a study performed by Aizatul et al. (2021), they highlighted the utilization of xylan. Xylan was highly used in coatings, binding and packaging, and also works well as an oxygen barrier. Even so, xylans from *Chlorophyta* sp. have not been well characterized as less research is concerning on this species.

Proteins and Amino Acids

Proteins and amino acid content in seaweeds differ from one another. According to the previous studies, *Chlorophyta* sp. recorded higher protein contents, which were 10 - 47 % of the dry weight (Notowidjojo, 2021). A study highlighted *U. lactuca* exhibited a relatively high protein content (6–32 % of the dry weight) compared to that of other species (Pangestuti & Kim, 2015). The protein contents in *C. racemosa* and *U. fasciata* were 8.8–19.9 % and 8.0–11.1 %, respectively (Magdugo et al., 2020). The protein content variation in Chlorophyta sp. has been influenced by the habitat and depth of the seaweed area. Similarly, U. lactuca recorded a higher protein content (19 % of the dry weight) than that in wheat (14.2 % of the dry weight) (Van der Heide, Stødkilde, Nørgaard & Studnitz, 2021). Hence, this species may become a suitable substitute for wheat in extracting a remarkable amount of protein content. Furthermore, Chlorophyta sp. do not compete with higher plants for space and resources; thus it may become the most significant alternative protein source in the future. Moreover, various protein extraction methods concerning this species have already been performed, such as enzymatic hydrolysis and ultrasonic-assisted extraction, which will benefit the public in consuming this species.

On top of that, Chlorophyta sp. was reported to be rich in essential (arginine) and non-essential amino acids (glycine, alanine and glutamic acid) (Ganesan et al., 2020). Caulerpa was also recorded to afford the highest amounts of essential and non-essential amino acids among the other green seaweeds. The essential amino acids, namely leucine, lysine, methionine, phenylalanine and valine, content were 0.2, 0.5, 0.4, 0.2 and 1.3 mg/g protein, respectively (Tanna, Brahmbhatt, & Mishra, 2019). Not to mention, the histidine and taurine content in Ulva and Caulerpa also play a vital role in fetus development (Ganesan et al., 2020). In contrast, U. pertusa accommodated a remarkable amount of both essential and non-essential amino acids, which were arginine and glycine (Ganesan et al., 2020). Arginine and glycine are involved in the metabolic pathways, regulation of intestinal function, and protein synthesis and performance (Barekatain et al., 2019). A known food source for essential amino acids such as lysine and histidine is usually acquired from meat, eggs, dairy cheese, soy, fish, and fish products. Canned sardines, mackerel, tuna and marinated anchovies showed an exceptional content of biogenic amines (histamine, tyramine, tryptamine, putrescine and cadaverine), ranged from 26.58 to 406.55 mg/kg. These biogenic amines were formed from the free amino acids histidine, tyrosine, tryptophan and lysine (Bilgin & Gençcelep, 2015). Attia, Al-Harthi, Korish, and Shiboob (2020) discussed the total essential and non-essential amino acid contents in eggs from Jeddah, Saudi Arabia. The eggs contained different percentage of essential (arginine, histidine, isoleucine, leucine, lysine, methionine, methionine + cysteine, phenylalanine, threonine, tryptophan, valine) and non-essential amino acids (alanine, aspartic acid, glutamic acid, glycine, proline and serine), which were 56.34 and 70.33 mg/g, respectively (Attia et al., 2020). Although total amino acid contents were higher in eggs

and others than those in green seaweeds, several strict vegetarians do not consume eggs, dairy cheese and any other product derived from animals. Hence, this species can be exploited in the future to become an alternative or a potential source of food proteins and amino acids.

Lipids and Fatty Acids

Seaweeds were reported to contain low lipids content (Pati et al., 2016). Nonetheless, it is a good source of polyunsaturated fatty acids (PUFAs) compared to other foods derived from plant and animal sources. PUFAs manifested a significant part in regulating blood clotting, blood pressure, brain and nervous system (Notowidjojo, 2021). Nevertheless, a high level of PUFAs was observed from a cold-water geographical region where the *Chlorophyta* sp. lies; meanwhile a high level of saturated fatty acids and oleic acid was recorded from a warm water Chlorophyta sp. (Notowidjojo, 2021). This can be deduced that lipids and fatty acids composition vary between geographic regions. According to Pati et al. (2016), E. clathrata from Chlorophyta exhibited the highest lipid content of 4.6 %, followed by C. tomentosum (2.53 %).

Meanwhile, low lipid content has also been noted in E. intestinalis (1.33%) and U. lactuca (1.6%) (Pati et al., 2016). Besides, lipid content recorded in U. rigida collected from Chillka Lake, India, was 12 %, which was the highest compared to previously mentioned lipid content in other seaweeds species (Satpati & Pal, 2011). On the contrary, the lipid content in fish displayed a varied range, usually from 0.2 to 25 % content and exhibited a less amount of lipid and fatty acid composition than those in red meat (Pal, Shukla, Maurya, & Verma, 2018). In addition, the lipid and fatty acids composition in fish is highly dependent on the species and seasons, which might be a disadvantage in acquiring sufficient lipid and fatty acid in the future. In the recent research, a study on the fatty acid compositions in quinoa was investigated. The main fatty acids detected were linoleic acid, oleic acid, palmitic acid, and α -linoleic acid (Pellegrini et al., 2018). Overall, the fatty acid content in quinoa comprised 4.87 to 6.48 g/100 g, which also showed a higher content when compared to that of *Chlorophyta* sp (Pellegrini et al., 2018). Despite that, *Chlorophyta* also contains α -linolenic acid, also known as an omega-3 fatty acid that plays a vital role in human physiology (Hasan, 2017). Therefore, it can be manifested that different seaweed species and habitats accommodated a great variation of total lipid and fatty acid contents, which might be advantageous for human consumption rather than foods that are derived from other plant and animal sources.

Pigments

Chlorophyta carried a photosynthetic pigment pattern that is almost analogous to the higher plants. Several photosynthetic pigments are present in *Chlorophyta*, namely chlorophyll a, chlorophyll b and, carotenoids.

Chlorophylls

The most common types of chlorophylls present in Chlorophyta are chlorophyll a and chlorophyll b. *Chlorophyta* utilized these photosynthetic pigments to absorb light needed for the growth, with the presence of carbon dioxide and carbohydrates. Chlorophylls contained reduced porphyrin rings, with a central magnesium atom and a long hydrophobic tail, making them less soluble in water (Martins et al., 2021a). In most industries, chlorophylls have been used as natural colorants in foods and beverages and been reported to portray antioxidant, antitumor and antimicrobial activities (Martins et al., 2021a). Martins et al. (2021b) revealed that a maximum yield of chlorophyll (5.96 mg/g DW) was successfully extracted from U. rigida, using a cost-effective extraction method, using 250 mM tributyl(tetradecyl)phosphonium chloride as the solvent.

Carotenoids

The main carotenoid compounds found in Chlorophyta were β -carotene, lutein, neoxanthin, antheraxanthin, zeaxanthin and violaxanthin. The latter was only present in *Chlorophyta* sp. (Othman, Amin, Sani, Fadzillah, & Jamaludin, 2018). These carotenoids present absorbed light at different wavelengths than chlorophyll, allowing the species to acquire some light to survive in the low sunlight conditions (Martins et al., 2021). Present studies recorded that C. lentilifera had the highest zeaxanthin and β -carotene contents 21.3 and 10.7 µg/g DW, respectively, compared with other seaweed species (Othman et al., 2018). Similarly, Aditi, Jaishini, Raisa, Aamna, and Rupali (2020) reported that carotenoids were identified for green microalgae; Spirogyra neglecta, Pithophora oedogonia and Microspora indica, based on Thin-layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC) analysis. U. lactuca also presented a significant content of carotenoids in which β -carotene recognized as major compounds (11.44 – 11.47 %) (Abd El Hafez, Elkomy, Saleh, and Aboul-Ela, 2020). This promising total amount of pigment contents in Chlorophyta make it a perfect target for use as a colorant, antioxidant, antimicrobial and Active Pharmaceutical Ingredient (API) in the future. However, only a handful of studies had reported efficient

purification processes of the pigments at a high purity level required by most industries.

Minerals

Seafood, including seaweed species, are usually known to hold an abundance of minerals such as iodine, magnesium, calcium, phosphorus, iron, potassium, copper and fluoride (Gokulkrishnan, Anantharaman, Manivannan, Thirumaran, & Balasubramanian, 2011). These minerals play a significant role as a cofactor of the enzyme in the human body. For instance, calcium and magnesium help maintain the bone and teeth strength, while sodium and potassium involve in the transfer of nutrients (Gokulkrishnan et al., 2011). Hence, it is believed that seaweeds are valuable for human consumption, as studies showed that seaweeds have great minerals content. U. reticulata presented the maximum contents of chromium, copper and magnesium. At the same time, Halimeda tuna only exhibited low mineral contents of cobalt, iron, magnesium, manganese, nickel, lead and zinc (Gokulkrishnan et al., 2011). Besides, C. lentillifera from the Kei Islands of Indonesia contained magnesium, potassium and zinc, whereas C. lentillifera from the Seribu Islands contained calcium (119.20 g/kg), sodium (34.18 g/kg) and iron (0.34 g/kg), respectively (Tapotubun et al., 2020). Also, a study on C. taxifolia from the shores of Kanyakumari, India yielded the mineral contents of copper (9.1 \pm 0.017 µg g⁻¹ dry weight), zinc (19.96 \pm 0.115 µg g⁻¹ dry weight), manganese (53.05 \pm 0.058 µg g⁻¹ dry weight) and chromium (5.15 \pm 0.087 µg g⁻¹ dry weight). However, the variation of total mineral content was observed when the seaweed was extracted for their mineral contents during two different months, April and December (Sethi & Karmegam, 2020).

Secondary Metabolites

Chlorophyta is also known to become a main source of secondary metabolites, which benefits humans in combating various pathogens (Levasseur, Patrick, & Victor, 2020). According to recent research, *Ulva* and *Caulerpa* have shown multiple bioactivities such as antibacterial, antitumor, antiviral and anti-inflammatory due to the presence of secondary metabolites (Shah et al., 2020). Among the secondary metabolites available in *Chlorophyta* are phenols, alkaloids and terpenes. *Caulerpa* was one of the genera reported to contain phenolic compounds (tannins and flavonoids), terpenes and steroids (Shah et al., 2020).

Alkaloids

Alkaloids is a cyclic organic compound with a nitrogen-containing base, which also exhibited a diverse

and vital physiological effect on humans. The physiological effects exerted were owing to the nitrogen found in the molecular structure of alkaloids. Alkaloid compounds, namely bisindole alkaloids and caulerpine, were mainly isolated from Caulerpa. The novel bisindole alkaloids, racemosins A and B, were successfully extracted from *Caulerpa* collected from Zhanjiang coastline in the East China Sea, China. This alkaloid compound was believed to show a neuroprotective action (Shah et al., 2020). In contrast, caulerpine and caulerpic acid from Caulerpa demonstrated anti-inflammatory and antinociceptive (Shah et al., 2020). Cantarino, Coutinho, Soares, Duarte, and Martinez (2020) varied the extraction method of caulerpin from C. racemosa. The highest caulerpin content (32.06 %) was recorded from the Soxhlet extraction method, followed by the maceration technique (19.50 %), microwave-assisted extraction (17.70%) and ultrasonic-assisted extraction (11.84%), respectively. Therefore, it can be said that the alkaloid contents may be varied depending on the type of extraction techniques used.

Terpenes

Other than alkaloids, terpenoids are one of the primary secondary metabolites widely found in *Chlorophyta*. Terpenoids can be categorized as mono, sesqui-, di-, sester-, tri- and tetraterpenoids based on the number of isoprene units. *C. taxifolia* contained sesquiterpenes (three units of isoprene, the backbone of the C15 carbon) with an unusual aromatic carbon skeleton of valerenance type (caulerpals A and B) (Shah et al., 2020). These metabolites, caulerpals A and B, were responsible for inhibiting human protein tyrosinase phosphatase. Meanwhile, Chakraborty & Santra (2008) studied the diterpenoids (four units of isoprene, the backbone of C20) in *U. fasciata*, which has been assumed to exhibit an antibacterial effect (Shah et al., 2002).

Phenols

A phenolic compound is defined as a group of small molecules that bearing at least one phenol unit. It can be divided into different subgroups based on its chemical structure, namely phenolic acids, flavonoids, tannins, coumarins and a few more. *Chlorophyta* exhibited a unique content of phenolics and flavonoids compounds. Al-Malki, Barbour, Al-Zahrani, and Moselhy (2018) studied the different total phenolic and flavonoid contents in *U. lactuca*. The phenolic content ranged between a minimum mean of 38.9 GAE/g when extracted using chloroform. The highest total phenolic content was observed at a maximum mean of 77.3 GAE/g when treated with ethyl acetate solvent.

Similarly, the total flavonoid content was recorded at 31.2 and 60 mgQE/g when recovered by chloroform and ethyl acetate solvents, respectively (Al-Malki et al., 2018). For this reason, it can be disclosed that the ethyl acetate solvent will significantly increase the yield of phenolic and flavonoid contents in other seaweed species in the comparative research. A wide range of antioxidant byproducts may be well established from the tabulation of the total phenolic compounds in *Chlorophyta*.

Vitamins

Sufficient and good nutrition is the key to a healthy lifestyle. Intake of nutritious food keeps diseases at bay, apart from enhancing the well-being of a human. Nutritional deficiency often occurs among the elderly, which may cause them to suffer from nutritional anemia, such as iron, folate and vitamin B12 (Shahar, Budin, Bakar, Umar, & Halim, 2005). Besides, the world has reported multiple issues related to health, such as vitamin deficiencies that affect all ages, including pregnant and lactating women. Strict vegetarians are more susceptible to vitamin B12 deficiency since they limit meat consumption (Bo et al., 2019). As stated by Ganesan, Tiwari, and Rajauria (2019), algae contain the highest amount of both essential and non-essential vitamins. For example, vitamin B12 content is higher in microalgae Chlorella, which was 33.3 µg/kg fresh weight than macroalgae nori (1 µg/kg fresh weight). However, nearly 60 % of active vitamin B12 aggregated coenzymes from the macroalgae nori would cover the daily need of biologically active vitamin B12 if it is fortified in a smoothie (Ganesan et al., 2019). Subramanian, Manivannan, Sona, Ravi, and Sasikala (2015) highlighted the vitamins A, B1 and B2 contents in U. rigida (vitamin A: $> 0.6 \pm 0.12$ mg/kg, vitamin B1: 5.85 \pm 0.04 mg/kg, vitamin B2: 1.22 \pm 0.01 mg/ kg) and U. lactuca (vitamin A: $> 0.5 \pm 0.11$ mg/kg, vitamin B1: 5.22 \pm 0.06 mg/kg, vitamin B2: 0.97 \pm 0.01 mg/kg), respectively. Despite the lack of studies on vitamin content in green seaweed, the outcomes revealed that the seaweeds can become a source of nutrients and as an alternative source of several vitamins.

Biological Activities of Chlorophyta

In terms of biological activities, green seaweeds have been reported to exhibit antimicrobials, antioxidants, antiviral, anti-obesity, anti-inflammatory and immunostimulatory properties. These properties are essential to suppress the effects of various diseases.

The antimicrobial activity in *U. lactuca* has been found to be effective in controlling human pathogenic microorganisms (Yu-Qing, Mahmood, Shehzadi, &

Ashraf, 2016). The U. lactuca extract was tested against some human pathogenic bacteria, such as Salmonella paratyphi, Pseudomonas aeruginosa, Vibrio cholera, Staphylococcus aureus, Shigella dysentriae and Klebsiella pneumonia, yet the active compound responsible for the antimicrobial activity was not mentioned. This antimicrobial activity was tested using the inhibition zone method. As a result, 11.2 mm of inhibition zone was noted for the extract against P. aeruginosa (Vallinayagam, Arumugam, Kannan, Thirumaran, & Anantharaman, 2009). Also, a review done by Zamimi, Halim, Mustafa, Darnis, and Musa (2020) discussed the antimicrobial activity of the C. lentillifera and C. racemosa extracts against S. aureus, which the minimum inhibition concentration (MIC) values recorded were about 125.25 to 375.75 mg/mL, respectively. The same inhibitory activities against S. aureus also were observed when C. cuppressoides extract was introduced, which the inhibition zone was 6 mm. This Chlorophyta sp. possesses these antimicrobial properties. mainly due to the presence of fatty acids, polysaccharides, and pigments (Zamimi et al., 2020).

On top of that, polysaccharides from seaweeds can also become a potential source of antioxidants. Antioxidants scavenging free radicals exhibited a significant role in preventing reactive oxygen species (ROS)-induced diseases such as carcinogenesis, cardiovascular disease, Alzheimer's disease, aging and neurological disorders (Li et al., 2020). As reported in their studies, the high sulfate content purified ulvan, extracted from U. pertusa, successfully improved the antioxidant activity in mice. Moreover, oligosaccharides from U. lactuca demonstrated an anti-aging effect when applied to Senescence Accelerated Mouse-Prone 8 (SAMP8) mice. It was observed that the total antioxidant capacity of the mice increased. Hence, it can be deduced that oligosaccharides from U. lactuca can be utilized in the making of nutraceutical products and in cosmeceutical to prevent aging problems (Liu et al., 2019). In addition, Abd El Hafez et al. (2020) reviewed the antioxidant activity possessed by U. prolifera by the total antioxidant capacity method. From their studies, the total antioxidant capacity exhibited by different solvent extracts, namely hexane, ethyl acetate and methanol extracts, were 0.97 ± 0.09 , 1.23 \pm 0.04 and 1.63 \pm 0.09 mg equivalent ascorbic acid/g dw, respectively, which was due to the polysaccharide, lipid, and protein contents in U. prolifera (Abd El Hafez et al., 2020). Additionally, the pigment contents in Chlorophyta can be beneficial in acting as antioxidants as pigment-like carotenoids can inhibit active radicals (Hidayati, Yudiati, Pringgenies, Oktaviyanti, & Kusuma, 2020).

Seaweed species, particularly Chlorophyta, are also known for their ability against viral infections; hence this species can be exploited to formulate some antiviral drugs. As reported by Mattos, Romanos, de Souza, Sassaki, and Barreto-Bergter (2011), the incorporation of seaweed species in the antiviral drug formulation now aims to investigate their activities against Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV). Few reports described several antiviral activities against HSV1, Japanese encephalitis virus (JE) and White Spot Syndrome Virus (WSSV) of the polysaccharides from Ulva sp. (Sun et al., 2018). Moreover, there is also a review done by Riccio et al. (2020), which analyzed the antiviral activity showed by sulfated polysaccharides extracted from U. pertusa, by targeting Avian Influenza virus (AIV) particle attachment to the cells. Aguilar-Briseño et al. (2015) reported the ulvan compounds from U. clathrata possessed the ability to target the Newcastle disease virus (NDV) by inhibiting the cell-cell fusion via a direct effect on the F0 protein. Furthermore, ulvan extracted from Enteromorpha sp. targeted WSSV, yet the action mechanism was not reported (Riccio et al., 2020). A study was conducted on the antiviral treatment of Zika virus (ZIKV), which presented the inhibition of C. racemosa towards ZIKV replication in a dose-dependent manner. The study found that C. racemosa extract exhibited the highest cytotoxicity (CC₅₀) effect, 732 µg/mL against ZIKV (Cirne-santos et al., 2017). It can be said that *Chlorophyta* extracts can be a promising species for further studies for the development of new antiviral agents.

Not only that, Chlorophyta, specifically C. okamurae are rich in active compounds such as minerals, fiber, vitamin A, vitamin C, alkaloids, β sitosterol and essential unsaturated fatty acids that was reckoned to possess an anti-obesity effect. A study done on 6-week-old male C57BL/6J mice exhibited a significantly lower body weight when supplemented with 250 mg/kg body weight of C. okamurae group compared to a mouse fed with high fat diet (HFD) alone. It was observed that the weight of epididymal and perirenal adipose and the total fat of the mouse were decreased when fed with C. okamurae diet (Gómez-Zorita et al., 2020). Given these studies, it can be deduced that C. okamurae extract has the potential to prevent obesity. This is because C. okamurae supplementation reduced the protein expressions of peroxisome proliferator-activated receptor-gamma (PPARy) and CCAAT/enhancerbinding protein alpha (C/EBP α) that increased due to the HFD (Gómez-Zorita et al., 2020). Similarly, Sharma, Kim, Kim, Park, and Rhyu (2017) also highlighted the anti-obesity effect demonstrated by C. okamurae extracts. It was found that the ethanolic

extract from *C. okamurae* was successfully inhibited the lipid accumulation, reduced the expression of PPAR γ , sterol regulatory element-binding protein-1c (SREBP1-c) and C/EBP α in 3T3-L1 adipocytes of a HFD-fed mice (Sharma et al., 2017).

Extract from Ulva sp. also exhibited a promising anti-inflammatory activity. McCauley, Winberg, Meyer, and Skropeta (2018) discussed the lipid-rich extract of Ulva, which had been cultivated under high nutrient conditions, possessed anti-inflammatory activity. This study resulted that Ulva sp. exhibited a strong antiinflammatory activity of > 94 %, as well as no cell toxicity (McCauley et al., 2018). Other than that, the low dose of sulfated polysaccharides (1 mg/kg) from U. lactuca also presented an anti-inflammatory effect. The sulfated polysaccharides content inhibited osmotic edema, that is characterised by bradykinin action (de Araújo et al., 2016). It is indicated that the structural compound from sulfated polysaccharides from U. lactuca plays a role in controlling these antiinflammatory pathways. Another study was done on the U. lactuca hydroethanolic extract, which worked as an anti-inflammatory agent in suppressing rheumatoid arthritis in male Wistar rats. The improvement was observed on the arthritis rats after three weeks of orally treated with a dose level of 100 mg/kg weight of U. lactuca hydroethanolic extract (Ahmed, Soliman, Mahmoud, & Gheryany, 2017). The administration of hydroethanolic extract of U. lactuca improved the elevated level of the rheumatoid factor (RF), prostaglandin E2 (PGE2), tumor necrosis factoralpha (TNF- α), interleukin-17 (IL-17), and interleukin-1-beta (IL-1b), also the lowered interleukin-4 (IL-4) level (Ahmed et al., 2017).

Moreover, bioactive compounds from Chlorophyta sp. also exhibited an immunostimulatory activity. Berri et al. (2017) discussed an immunostimulatory activity of sulfated polysaccharides extracted from U. armoricana. The capacity of extracted ulvan from U. armoricana to act as immunostimulatory was examined on the gut using an in vitro system of porcine intestinal epithelial (IPEC-1) cells. It showed that the mRNA and protein expression of cytokines such as CCL20, IL8, and TNF- α was significantly increased with the concomitant of ulvan extract (Berri et al., 2017). Besides, the galactans compound extracted from C. cupressoides was also reported to possess immunostimulatory activity. It was reported that the production of nitric oxide, reactive oxygen species and proinflammatory cytokines TNF- α and IL-6 were increased with the presence of C. cupressoides extracts containing galactans (Da Silva Barbosa et al., 2020). Overall, it can be deduced that various species from *Chlorophyta* sp. may exhibit immunostimulatory activity with potential therapeutic applications. Table 1

summarizes the bioactive compounds of some *Chlorophyta* species with their important biological activities.

Application of Chlorophyta in Industries

Chlorophyta has gained keen interest by two primary industries, cosmeceutical and nutraceutical industries. In general terms, cosmeceutical is referring to a cosmetic product with medicinal or drug-like advantages. Cosmetics are routinely used by most individuals today since many outdoor activities are being practiced throughout the day. Thus, extra care needs to be practiced to protect the skin from external stimuli. The external stimuli may be direct exposure from the ultraviolet coming from the sun, the dust from the surrounding, and many more. Despite that, Chlorophyta has also been widely incorporated as dietary supplements in the nutraceutical industries. The purpose of adding a dietary supplement into the regular diet is to boost and enhance the diet with a nutritional and physiological effect. In general, the nutraceutical is defined as any food or part of food that caters to medical or health benefits and acts as a tool in preventing diseases. Examples of nutraceuticals are natural foods, which include antioxidants, dietary supplements, fortified dairy products, vitamins, minerals, cereals, herbals and milk.

Incorporation of *Chlorophyta* in Cosmeceutical Industries

People have recognized a traditional cosmetic with a more natural composition in this age, as the value of applying a healthy and safe cosmetic has been highlighted. A healthy cosmetic must be used to avoid any side effects that can cause toxicity to the user. Several studies have reported adverse side effects such as contact dermatitis and allergic reactions when applying certain cosmetics that contain high concentration, exceeding the optimum limits of bioactive substances (Panico et al., 2019). In this context, it is crucial to invent a new whole cosmetic product that is consumer-friendly to prevent these unwanted reactions. Thus, seaweed species such as Chlorophyta were exploited in the cosmeceutical industries in manufacturing a new line cosmetic product which will act as a thickening, gelling agent and many more. Generally, the incorporation of seaweed species stimulates the extracellular tissue matrix (ETM) production by increasing the neocollagenesis, thus enhancing the consumer youth and well-being and the skin replacement (Pimentel, Alves, Rodrigues, & Oliveira, 2018). The most common cosmeceutical products containing seaweed extracts included creams, moisturizers, serums and lotions.

Biological activities	Species	Bioactive compounds	References
Immunostimulatory	U. lactuca,C. lentilifera,C. racemosa,C. cuppressoides, U. pertusa, U. prolifera	Fatty acids, polysaccharides, pigments, galactans	Yu-Qing et al. (2016); Vallinay agam et al. (2009); Zamimi et al. (2020)
Antioxidants Anti-inflammatory	U. pertusa, U. lactuca, U. prolifera, U. armoricana, U. rigida U.lactuca, U. rigida, C. racemosa	Polysaccharides; mainly ulvans, oligosaccharides, lipids, proteins, carotenoids, chlorophyll a, chlorophyll b Polysaccharides; mainly ulvans	Shah et al. (2020); Figueira et al. (2020) Shah et al. (2020)
Antimicrobial	U. rigida, C. racemosa	Fatty acids, polysaccharides, pigments, galactans, chlorophyll a, chlorophyll b, terpenes	Yu-Qing et al. (2016); Vallinay agam et al. (2009); Zamimi et al. (2020), Martins et al. (2021), Shah et al. (2020)
Antiviral	Ulva sp., U. pertusa, U. clathrata, C. racemosa	Polysaccharides; mainly ulvans	Figueira et al. (2020)
Anti-obesity	C. okamurae	M inerals, fibers, vitamin A, vitamin C, alkaloids, fatty acids,	Gómez-Zorita et al. (2020); Sharma et al. (2017)
Synthesis of protein, involved in metabolic pathways	Ulva sp., Caulerpa sp.	Proteins; arginine, glycine	Ganesan et al. (2020); Barekatain et al. (2019)
Fetus development	Ulva sp., Caulerpa sp.	Proteins; histidine, taurine	Ganesan et al. (2020)
Neuroprotective action	Caulerpa sp., C. racemosa	Alkaloids; bisindole alkaloids, caulerpine, racemosin A and B, Terpenes; diterpenoid	Shah et al. (2020);

Table 1. Bioactive compound of some Chlorophyta species with their promising biological activities

Polysaccharides from Chlorophyta, particularly ulvan and its highly sulfated derivatives, have been reported to possess radical scavenging activity, reducing power and metal chelating ability, which are useful in synthesizing certain chemicals (Priyan et al., 2019). Ulvan, which contains polyaldobiuronan has been used to synthesize aromas, producing strawberry odor and flavoring agents such as furaneol. This was due to the polyaldobiuronan moieties rich in the 6-deoxyhexose sugar rhamnose (Priyan et al., 2019). In addition, Lahaye and Robic (2007) discussed the synergistic skin protective and bioactive effects exhibited by rhamnose and fucose in combating skin aging problems. Similarly, mannans and xylans possess desirable properties to be used in the manufacturing industries. For instance, glucomannan has been incorporated in cosmeceutical as an emulsifier (Srivastava & Kapoor, 2005).

Chlorophyta is also known to contain a sustainable source of amino acids and peptides, where the cholorophyta peptides have a potential to protect collagen stores and enhance collagen synthesis (Palareti

et al., 2016). For instance, U. lactuca has been reported to revive collagen synthesis in human fibroblasts due to tripeptide containing an arginine-glycine-aspartic acid sequence (Palareti et al., 2016). Similarly, the peptides from C. vulgaris have shown the ability to reduce the matrix metalloproteinase-1 (MMP-1) expression in human skin cell fibroblasts responsible for the collagen breakdown (Chen, Liou, Chen, & Shih, 2011). In this case, the fibroblast is vital in human skin as its role is to repair and remodel the dermis during the skin antiaging process (De Araújo, Lôbo, Trindade, Silva, & Pereira, 2019). Therefore, the peptides from Chlorophyta may be capitalized in manufacturing a cosmeceutical product that will help prevent skin aging problems. Apart from that, a study conducted by Nurjanah, Nurilmala, Hidayat, and Sudirdjo (2016) discussed the amino acid contents in Caulerpa could be utilized as cosmetics material. The concentration of amino acid contents such as glutamate, histidine, arginine, aspartate, tyrosine, alanine and valine was high, above 100 mg/100 g. This amino acid content is vital in skin regeneration and in maintaining healthy skin.

Moreover, mycosporine-like amino acids (MAA) also was found abundant in green macroalgae and seemed to be potential cosmetic agents. The example of MAA family included mycosporine-glycine (MGly), palythine, palythinol, asterina-330, porphyra-334 and shinorine (Berthon et al., 2017). These compounds carried significant chemoprotective effects in preventing photoinduced skin aging.

Other than that, carotenoids of green seaweeds can also become sources of ingredient in the cosmetic industry anti-inflammatory, anti-aging, antiphotoaging, colorants and radical scavengers' properties (Christaki, Bonos, Giannenasa, & Florou-Paneria, 2013). βcarotene, for instance, can revitalize and enable the skin to fight against skin aging and reduce the risk of skin cancer among the users (Joshi, Kumari, & Upasani, 2018). Furthermore, β -carotene from U. lactuca acidic extract (155 x 10 g/L) exhibited antioxidant properties analogous to the commercial antioxidants and had a skin-irritant effect of 0.1 % (Balboa et al., 2014). Thus, it was safe for topical use in cosmetics. Consequently, U. reticulata crude extract had been formulated into an anti-aging serum formulation (Septiyanti, Liana, Sutriningsih, Kumayanjati, & Meliana, 2019). Joshi et al. (2018) also reported that the industry incorporated astaxanthin obtained from Haematococcus pluvialis in cosmetics, food and beverages.

Other than polysaccharides and pigment, *U. lactuca* has been reported to be abundantly incorporated in the cosmetic industry as an anti-wrinkle agent since this species contained a remarkable amount of several vitamins and minerals such as vitamin A, B, C and E, magnesium, iron and amino acids (£êska, Messyasz, & Schroeder, 2018). Therefore, it can be inferred that *Chlorophyta* sp. can be a promising source in cosmeceutical industries. Several other species are believed to be a promising source in cosmeceutical industries, as presented in Table 2.

Incorporation of *Chlorophyta* in Nutraceutical Industries

Chlorophyta species presented different nutritional values based upon their natural characteristics. The biochemical compounds originated from *Chlorophyta* offered many nutraceutical benefits. For instance, *U. fasciata* and *C. racemosa* contain a high concentration of polysaccharides, lipids and amino acids (Magdugo et al., 2020). In addition, Magdugo et al. (2020) reviewed that *Ulva* and *Caulerpa* were consumed directly by humans in the Philippines and have long been listed as nutraceutical products by the Food and Agricultural Organization of the United Nation (FAO) for its various health-promoting benefits.

Moreover, carotenoids from Chlorophyta have also been utilized in nutraceuticals, as well as in pharmaceuticals. Carotenoids may become an effective antioxidant; thus, it will be beneficial for human health. In terms of nutraceuticals, carotenoids such as tocopherol were used as a food preservative in some food products (Shah et al., 2020). Astaxanthin from H. pluvialis has also been reported to lessen inflammation, oxidative stress and enhance the immune system of patients who were suffered from cardiovascular disease (Shah et al., 2020). Apart from the nutraceutical mentioned above benefit of Chlorophyta species, Chlorophyta species, dried green seaweed (Enteromorpha) has been expected to be an alternative for vitamin B12, especially to those who are on a special diet. U. lactuca species is also a vital source of vitamin B (Macartain, Gill, Brooks, Campbell, & Rowland, 2007). They revealed that a daily intake of 1.4 g/day of U. lactuca is sufficient to meet the daily requirement of vitamin B12. Therefore, the algal species is a promising species that acts as an alternative source of vitamins in the future, especially to older people and strict vegetarians, which later can be exploited in the nutraceutical industries.

Tanna and Mishra (2018) had reported few Chlorophyta species were commercially available as nutraceutical products in a company located in Vietnam. They reported that the commercialized Ulva was rich in docosahexaenoic acid (DHA), with an omega ratio in the range of 0.61 to 5.15:1. Moreover, World Health Organization (WHO) recommended an 66/63 ratio of < 10 for nutraceutical to exhibit an ability to suppress neurological, inflammatory, and cardiovascular disorders (Tanna & Mishra, 2018). U. fasciata also exhibited an anticoagulant effect due to the presence of galactans and fucans (Ruocco, Costantini, Guariniello, & Costantini, 2016). Overall, the bioactive compounds in seaweed, especially in green seaweed species, offered many nutraceutical benefits to human health. Table 3 summarizes the application of several species from Chlorophyta in terms of their current and future nutraceutical properties and products.

Conclusion

In short, seaweeds offered numerous nutrients to benefit human beings. Seaweeds, particularly *Chlorophyta* are rich in beneficial compounds such as polysaccharides, proteins, amino acids, fatty acids, minerals, and vitamins with medicinal and healthpromoting effects. Therefore, this nutritional value makes *Chlorophyta* a valuable future food supplement and a precious component as cosmetic ingredients. *Chlorophyta* also benefits those on a strict diet or vegetarians due to excluding certain nutrients or

Cosmetic properties/products	Species	Extracts/Compounds	References
Antioxidant	E. linza, Bryopsis plumose, U. rigida	Polysaccharides	Pereira (2018); Thiyagarasaiyar, Goh, Jeon & Yow (2020)
Antioxidant – DDPH inhibitions	C. antennia	Fucoxanthin	Thiy agarasaiy ar et al. (2020)
Algotherm – soothing power, hydrating properties, exfoliating	U. compressa, Codium tomentosum	M agnesium, p oly saccharides	Michalak, Dmytryk, & Chojnacka (2020)
M oisturizing agents	U. rigida, U. lactuca	Polysaccharide ulvan, extracts	Priy an et al. (2019); Pereira (2018); Lahaye & Robic (2007); Pimentel et al.
Exfoliating gels, body masks, body scrubs, face peelings, face masks, cleansing gels	U. lactuca, U. compressa	Extracts, micronized algae	Pereira (2018)
Anti-stretch mark creams, body lotions, eye creams, face masks	C. vulgaris	Extracts	Pereira (2018)
Anti-inflammatory agent	U. lactuca	Poly saccharide ulvan, Carotenoids (astaxanthin, β- carotene, fucoxanthin,	Pimentel et al. (2018); Pereira (2018)
Stimulation of collagen production, increase the collagen	U. lactuca	Tripeptide: arginine, glycine, aspartic acid	Wang, Paul, & Luesch (2013)
Anti-aging, antioxidant, tyrosinase inhibitors, antiphotoaging agents, radical	U. lactuca	Carotenoids (astaxanthin, β- carotene, fucoxanthin, lutein)	Pereira (2018)
Antibacterial	U. lactuca	Chlorophylls	Pereira (2018)
Antiadhesive agents	Ulva sp.	Lectins	Pereira (2018)
Tyrosinase inhibitor to inhibit melanin pigment	Caulerpa sp.	Steroids, flavonoids, phenols	Pereira (2018)

Table 2. Applications and functions of extracts extracted from Chlorophyta sp. in the cosmetic industry

Table 3. Applications and functions of extracts from *Chlorophyta* sp. in the nutraceutical industry

Nutraceutical properties/products	Species	Extracts/compounds	References
Lettuce extracts, vegan alternatives to beef-derived gelatins	Ulva and Enteromorpha sp. U. lactuca, U. armoricana	Poly saccharide ulvan	Tanna & Mishra, (2018); Shannon & Abu-Ghannam, 2019
Nutraceutical product by Vietnam company	Ulva	Docosahexaenoic acid (DHA)	Tanna & Mishra, (2018)
Semi-sweet biscuit with antioxidant	C. racemosa	poly saccharides	Kumar et al. (2008)
Food stabilizer and preservatives	U. lactuca , U. pertusa , U. clatharata , U. intestinalis , U. linza	Bromophenols and flavonoids	Leandro et al. (2020)
Dietary supplements	Enteremorpha, U. lactuca	Vitamin B12 (Cyanocobalamin)	Macartain et al. (2007)
Healthy snacks in Thailand	U. rigida	Dietary fiber, proteins, minerals	Thunyawanichnondh et al. (2020)

minerals from their daily dietary intake, specifically vitamin B12. This problem is problematic as it can lead to nutrient deficiency that causes diseases. Moreover,

recognition of seaweeds species as a source of nutrients is crucial to suppress some illnesses related to nutrient deficiency among the elderly and others.

Seaweeds are becoming one of the most desirable natural sources for obtaining biological compounds due to their high potential for producing novel nutraceutical and cosmeceutical products. Therefore, further research is required to highlight the seaweed (Chlorophyta) aquaculture, its extraction method, and the most targeted bioactive compounds from the species. In addition, the preservation of the bioactive compounds during extraction, which is mostly unstable upon contact with oxygen, high temperature, and light, must also be considered so the extraction and aquaculture methods can be easily applied in the industries. In the current research, the focus is more on the cultivation method of seaweed that will give a similar or higher concentration of bioactive compounds, which correspond with the bioactive compounds from wild seaweed. Likewise, the optimization of the extraction method needs also to be further studied to yield better quality and quantity of the extract. Also, it is crucial to develop an optimized extraction method that will help industries obtain more than one compound to create value added to the products. This review has emphasized the biological compounds, biological activities, and application in the industries that might also benefit future researchers in improving Chlorophyta sp. cultivating methodology and optimizing extraction methods.

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