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Effect of Ultrasound Pretreatment on the Extraction Kinetic of Bioactive Compounds from Brown Seaweeds

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Seaweeds are marine benthic plants that grow abundantly in Sabah. Seaweeds are rich in bioactive compounds which is beneficial to human health. In this article, several main bioactive compounds, which are laminarin, alginate, phlorotannin, fucoxanthin, diterpene, carrageenan, fucoidan and fatty acid, bioactive protein, agar, carotenoid, sterol and ulvan in seaweeds and its health effects, will be reviewed from 2005 to present. Laminarin, alginate, carrageenan, ulvan, agar and fucoidan are polysaccharides present majorly in seaweed cell wall. Phlorotannin is polyphenol, diterpene is hydrocarbon and fucoxanthin is carotenoid in seaweeds. Lipids are present as sterols and fatty acids while bioactive proteins are also present in seaweeds.

Introduction

Sabah is the main seaweeds producer in Malaysia, especially in the Semporna area (Ahmad, Mohd Rosni Sulaiman, Saimon, Yee & Mantajun, 2012). Seaweeds or macro-algae are multicellular marine plants composed of thallus, stem and holdfast. Generally, seaweeds are categorized into three basic groups, namely Phaeophyceae (brown seaweeds), Rhodophyceae (red seaweeds), and Chlorophyceae (green seaweeds). In the research done by Ahmad *et al.,* (2012) seaweeds were found to contain carbohydrate, protein, fat, lipid and fiber. Furthermore, seaweeds are also rich in vitamin and mineral, but they have low fat content. Being an edible and nutritive plant, they have many usages; they can be used as human food, animal feed, biomass for fuel, fertilizers, cosmetics and wastewater treatment (Mchugh, 2003). In the past, seaweeds were harvested or cultured for phycocolloids production and for edible purpose; however, recently, the intention has switched to the investigation of the beneficial health effect induced by bioactive compounds in seaweeds.

Bioactive compounds are nutritional constituents that present in small quantity, but exhibit positive effect on health. Recently, bioactive compounds have gained more interests and have been applied in a wide range of fields, for examples, agrochemicals, nano bioscience, modern pharmacology, geo-medicine, plant science, cosmetics, food industry and others (Guaadaoui, Benaicha, Elmajdoub, Bellaoui & Hamal, 2014). The content of bioactive compounds in seaweed varies due to its growing conditions, for example, water temperature, salt content, nutrients and light (Kadam, Tiwati & O'Donnell, 2013). Examples of bioactive compounds include polysaccharides (alginate and laminarin), phenolic compounds (phlorotannin and flavonoids), omega-3 fatty acids, carotenoids (β-carotene, volaxanthin and fucoxanthin) and others. The generation of bioactive compounds in seaweeds is due to the adverse marine environment that seaweed grows in. In marine, seaweeds are exposed to ultraviolet (UV) radiation, osmotic stress, desiccation, light, temperature fluctuations, pollution, and high oxygen concentration. These conditions lead to the generation of strong oxidizing agents and free radicals. However, seaweeds do not seem to suffer from any photodynamic damage caused by the harsh environment (Ahmad *et al.,* 2012). This was supported by Fung Nazimah Hamid & Lu (2013), who stated that the presence of antioxidants in seaweeds is related to the exposure to strong light intensity and high oxygen concentration in marine environment. They mentioned that reactive oxygen species and other strong oxidizing agents are produced from seaweed to prevent photodynamic damage or oxidative breakdown of the structural component, for example, polyunsaturated fatty acids. Apart from that, Kadam *et al.* (2013) also stated that marine algae generate a wide range of natural bioactive compounds as second metabolites for UV radiation, stress and herbivore protection. This indicates that seaweeds have their own protective mechanisms and compounds such as antioxidants. These protective compounds, also known as bioactive compounds, are not generated for growing but for protection in seaweeds. Apart from being secondary metabolites in seaweeds,

they had found to exhibit beneficial effects on human health. Bioactive compounds had discovered to exhibit biological functions such as anti-thrombotic, prebiotic, antioxidant, anti-hypertensive, immunomodulatory, mineral binding and opioid agonistic properties (Ngo, Vo, Ngo, Wijesekara & Kim, 2012). Hence, they can be utilized in cosmetic products, pharmaceuticals and functional foods as an additional nutritive value (Wu, Lim, Wan Aida Wan Mustapha, Mohamad Yusof Maskat & Mamot Said, 2014). Previous studies have shown that bioactive compounds can manage diabetes, reduce cholesterol level, regulate appetite, treat obesity and reduce the risk of cardiovascular disease (Yusrizam Sharifuddin, Chin, Lim, & Phang, 2015 & Brownlee, Fairclough, Hall & Paxman, 2012). These beneficial findings had led to the extraction of bioactive compounds from seaweeds and manufacture into seaweed extract capsules as a daily dietary supplement.

Bioactive compounds in seaweeds

Laminarin

Laminarin is present in the cell wall of brown seaweed and acts as the storage for polysaccharide. It is comprised of 1,3-β-D-glucan with high sugar content but low uronic acids. It is a linear chain with 6-O-branching in the main chain and β-1,6-intrachain links on 1,3-β-D-glucopyranose residues. There are two types of polymeric chains in laminarin, known as G-chains and M-chains (Moroney, O' Grady, Lordan, Stanton & Kerry, 2015). The end of G chain is attached to glucose while mannitol for M-chain. It can be either water soluble or insoluble depends on the level of branching. Its weight is approximately 5 kDa, which make up 35% of the dry weight of seaweed (Kadam *et al.,* 2015). The structure of laminarin is different among different species in the aspect of ratio of 1,6- and 1,3-glycosidic bonds, degree of branching and degree of polymerization. For instance, in *Laminaria digitata,* laminarin has a linear backbone of β-1,3- linked D-glucose with β-1,6 linked side chains while *Eisenia bicyclis* has a linear chain of (1-3) and (1-6) links in the ratio of 2:1 (O'Sullivan *et al.,* 2010).

In research done by Gupta & Abu Ghannam (2011), laminarin had demonstrated to possess anti-coagulant, anti-bacterial, anti-viral, anti-oxidant, immunomodulatory, antithrombotic and anti-tumor properties. Molecular structure, monosaccharide constituents and degree and length of branching affect the antioxidant property of laminarin (Moroney *et al.,* 2015). A previous study by Neyrinck, Mouson & Delzenne (2007) revealed that laminarin treatment reduces nitrite, tumor necrosis factor-alpha and serum monocytes number and modulates intra-hepatic immune cells. Through their findings, they proposed that the immunomodulatory properties which include low production of inflammatory mediators and low employment of inflammatory cells in liver tissue might be due to the hepatoprotective effect of laminarin. They also predicted that laminarin influences immune cells, either indirectly through dietary fiber properties or directly on them.

Besides, it is consumed as dietary fiber as it is able to form complex structures that resist hydrolysis in the upper gastrointestinal tract. This is due to the fact that laminarin is stabilized by inter-chain hydrogen bonds. The resistance of fucoidan toward digestive enzymes has been displayed by Deville, Gharbi, Dandrifosse and Peulen (2007) in the study of the effect of laminarin on human gut after incubating it *in vitro* with human saliva and gastric, small intestine, pancreatic and colonic homogenates, and hydrochloric acid. Apart from that, it also showed that laminarin can affect the production of short chain fatty acid, especially butyrate, intestinal pH and mucus composition. Hence it can modulate the metabolism in intestine. Besides, it is totally fermented and it increased the production of propionate and butyrate, which acts as the main energy source for colonic cells to stimulate the growing of epithelial cell. Furthermore, they also concluded that it can influence the adherence and translocation of bacteria across the epithelial wall as it affects the mucus composition in rats in the lumen content and the intestinal wall of jejunum, cecum, ileum and colon after ingestion.

Fucoidan

In brown seaweed, fucoidan is found in the cell wall and in the cell. It is a sulfated polysaccharide with sulfates located at carbon-2 position and L-fructose units link predominantly by α-(1,2) linkages (Moroney *et al.,* 2015). Galactose, mannose, xylose and glucuronic acids might also present as residues in fucoidan. Structural heterogeneity and lack of regularity raise the difficulty in formulating the chemical structure of fucoidan. This results in the difficulty to determine the relationships between fucoidan's activity and its structure (Shan *et al.*, 2016). However, up to date, previous studies had revealed four structures of fucoidan, which are fucoglucuronan with α-L-Fucp or β-D-Xyl branching on carbon-2 or carbon-4 of 1,3- or 1,4-linked β-D-GlcpA backbone; fucogalactan with galactose and fucose branching at terminal of carbon-4 or carbon-2 of (1-6)- β-D-Galp and/or (1-2)- β-D-Manp backbone; fucan sulfate with sulfate ester branching at carbon-4 and carbon-2 on linear backbone of alternating 1,3- and 1,4-linked α-L-fucopyranose or 1,3-linked α-L-Fucp backbone and fucogluronomannan with α-l-Fucp substituted at C-3 of 1,2-linked α-d-Manp branching at alternating 1,2-linked α-d-Manp and 1,4-linked β-d-GlcpA backbone (Cong, Chen, Liao, Xiao, Wang, Qin, Dong, & Ding, 2016). Branches, quantity of sulfate groups, types of glycosidic linkages and its molecular weight affect its activities. Similar to other bioactive compounds, it has different structure and pharmacological activities in different species. It constitutes 5-20% dry weight of seaweeds with 40% of it

is sulfate esters (O'Sullivan *et al.,* 2010). It aids the linking of alginate and cellulose and at the same time, functions as cell organization.

Previous studies revealed that fucoidan exhibit anticoagulant, antithrombotic, antiviral, antitumor, immunomodulatory, antioxidant and anti-inflammatory properties (O'Sullivan *et al.,* 2010). Wang, Wu, Hsieh, Tsai, Yeh & Huang (2015) proved the antioxidant activity and antitumor property as it prevents the growth of human colon cancer cells. The antioxidant property of fucoidan is affected by its degree of sulfation, sulfate content and molecular weight (Moroney *et al.,* 2015). Fucoidan also has been reported to decrease the risk of gastric cancers by inhibiting *Helicobacter pylori* infection in stomach and prevent the proteolysis of gastric mucosa by gastric juice in stomach (O'Sullivan *et al.,* 2010). Apart from that, Shan *et al.* (2016) demonstrated that fucoidan extracted from *Fucus vesiculosus* inhibited α-glucosidase, which induced type 2 diabetes and decreased the hemoglobin A1c levels and fasting blood glucose level in mice. Through the findings, they proposed the utilization of seaweed extracted fucoidan as a promising treatment for type 2 diabetes mellitus. Furthermore, Dinesh, Menon, Hanna, Suresh, Sathuvan & Manikannan (2016) revealed that fucoidan might possesses anti HIV activity, hence can be used as an agent to depress HIV. Cong *et al.* (2016) suggested the potential of fucoidan as anti-angiogenic agent as the fucoidan extracts from *Sargassum fusiforme* successfully prevents the migration and tube formation in human microvascular endothelial cells. In addition, Park, Yeom & Hahm (2016) found that fucoidan increase the high density lipoprotein cholesterol level and decrease triglycerides, total cholesterol level in serum and low density lipoprotein cholesterol level in mice in their investigation on the effect of fucoidan on serum lipid levels and atherosclerosis. They concluded that fucoidan modulates the expression of key enzymes of cholesterol and triglyceride syntheses in liver through manipulation of SREBP-2 and hence, improves serum lipid levels. Zhao, Guo, Hu, Zhang, Xue, Xhang & Li (2016) stated that fucoidan, especially low molecular weight fucoidan might be used as an oral antithrombotic agent as it prevent the formation of arterial thrombosis in mice treated with electric shock. Last but not least, Yokota, Nomura, Nagashima & Kamimura (2016) demonstrated that administration of fucoidan prevents the inflammation and oxidative stress and induce plasma lipoprotein lipase activity in HFD-fed ApoE mice and concluded that it has anti-dyslipidemic and anti-atherosclerotic effects.

Alginate

The quality and quantity of alginate vary according to their species, type and age of tissues. Alginate is abundant in marine sources and make up approximately 40% of the dry matters in seaweed (Hu, Tao, Wang, Xiao & Wang, 2016). In brown seaweeds, alginate presents as the structural components in cell wall. It is a linear sulfated polysaccharide constituting of two hexauronic acids: β-D-mannuronic acid (M) and α-L-glucuronic acid (G) units (Vauchel, Kaas, Arhaliass, Baron & Legrand, 2008). It is make-up of three different blocks, one type of heteropolymer, an alternating M and G residues, called MG blocks, and two types of homopolymers, D-mannuronic acid blocks (MM) and L-glucuronic acid blocks (GG). The biological and physical properties of alginate are affected by M/G ratio and arrangement of M and G units along the linear polymer (Rahelivao, Andriamanantoanina, Heyraud & Rinaudo, 2013). Different types of seaweeds and different harvesting period will affect the M/G ratio (Kim, Choi, Kim & Oh, 2015b).

Alginate is a water soluble polysaccharide. However, it presents as an insoluble form of mixed counterions in seawater, mainly in calcium salt form and others, for instances, magnesium and potassium (Song, Pham, Seon & Woo, 2015 & Andriamanantoanina & Rinaudo, 2010). Hence, to extract alginate from brown seaweeds, it is necessary to convert all alginates to their soluble forms as sodium salt. The gelling property of alginate provides strength to cell wall of brown seaweeds. Gelation of alginate occurs either in the presence of multivalent cations or when pH is below pK_a of constituting acids in alginate solution (Hu *et al.,* 2016). The ability of alginate to gel is mainly contributed by the GG blocks (Rahelivao *et al.,* 2013). This is due to the fact that the binding sites for the formation of polyvalent are during the two G residues are located adjacent in the polymer (Dett, Strugala & Richardson, 2011). Hence, it can said tha the gelling capability of alginate depends on the G residues content.

Alginate has wide application in textiles, agriculture foods, paper, cosmetics and pharmaceuticals industries due to its colloidal properties (Vauchel *et al.,* 2008). In food industry, it has wide applications due to its thickening characteristic and gel-forming abilities in the presence of calcium (Fenoradosoa Ali, Delattre, Laroche, Petit, Wadouachi & Michaud, 2010). In biotechnology field, it has been widely used as drug delivery agent, cell encapsulation material, wound dressing material, surgical sponge, embolization agent and polymer film (Kim *et al.,* 2015b). It also uses in encapsulation and releasing of cells and medicine (Fertah, Belfkira, Dahmane, Taourirte & Brouillette, 2014). In pharmacological study, Viswanathan & Nallamuthu (2014) reported that alginate prevents chemically induced cancerogenes and ulcerogenes and lowers the blood sugar level. Besides that, alginate is also a treatment for obesity due to the gelling property that able to delay gastric emptiness and decrease the amount of intake of food (Hu *et al.,* 2016). The study done by Jensen, Kristensen & Astrup (2012) showed that the intake of alginate as dietary fiber causes significant weight loss in obese people. Houghton, Wilcox, Chater, Brownlee, Seal & Pearson (2015) proved

that the dietary function of alginate is not destroyed by heat. This is due to the result demonstrated that the inhibition of digestive enzymes *in vitro* retained even after cooking the alginate contained food at high temperature up to 150°C. They also reported that alginate undergoes either ionic gelation in the presence of multivalent cations or acidic gelation when the pH is lower than 3.5. As the pH in the stomach is low, gelation occurs and increases the viscosity of fluid in the stomach. As a consequence, the digestibility of macronutrients reduces which leads to reduction in hunger. Last but not least, Solah, Kerr, Adikara, Meng, Binns, Zhu, Devine & Prince (2010) reported the similar finding regarding the satiety effect after the intake of alginate drinks.

Apart from anti-obesity properties, Kuda *et al.* (2015) showed that sodium alginate can utilize to treat food poisoning. They found out that sodium alginate had reduced 70-90 % of pathogen adhesion in human enterocyte-like HT-29-Luc cells, prevented the invasion of *S. Typhimurium* and reduced the faecal pathogen count in mice. Besides that, Fertah *et al.* (2014) proposed the utilization of alginate in obesity and diabetes treatment based on its anti-hyperglycemic properties and inhibitory action on fat absorption. Alginate also has the potential to reduce the health problem caused by high rates of fat absorption such as hyperlipidaemia. This has been shown by Mackie *et al.* (2016) that the diffusion of lipid in emulsion digesta had reduced with an addition of 0.1% alginate to porcine intestinal mucus. Clinical studies conducted by Dett *et al.* (2011) revealed that the consumption of alginate did not reduce the amount of food consumed at a meal sitting but delay the onset of hunger, reduced the daily intake energy by 7 % and decreased the blood cholesterol and glucose levels in subjects with Body Mass Index more than 2.

Carrageenan

Carrageenan is the major phycocolloids present in the cell wall of red seaweed as sulfate polysaccharides in large quantities (Pangestuti & Kim, 2014 & Gomez-Ordoriez, Jimenez-Escrig & Ruperez, 2012). It is a linear chain composed of repeated units of disaccharide with alternating 3-β-D-galactopyranose and 4-3.6-anhtdrogalactopyranose or 4-α-D-galactopyranose residues (Diogo, Novo, Gonzalez, Ciancia & Bratanich, 2015). There are three types of carrageenan, known as kappa/iota, lambda and mu/nu carrageenans. They differ in terms of distribution, the proportion of sulfate groups and the presence or absence of 3,6-anhydrogalactopyranose. Each type of carrageenan then further divided into different groups according to their position and amount of (3,6) -anhydro-galactose content and ester sulphate substitutes (Ordoriez *et al.,* 2012). Lambda carrageenan is extracted from tetrasporic plants of Rhodophyta and has only trace amounts of 3,6-anhydrogalactose but a very high sulfate content. Kappa carrageenan is a disaccharide composed of 1,4-linked-3,6-anhydro-α-D-galactose and 1,3-linked β-D-galactopyranose-4-sulphate (Raman & Doble, 2015).

Pangestuti *et al.* (2014) reported that carrageenan has a wide range of pharmacological activities both *in vivo* and *in vitro*, for instances, antioxidant, anticoagulant, cholesterol lowering effect, antiviral, anti-tumor and immunomodulatory activity. Diogo *et al.* (2015) showed that lambda carrageenan can prevent the infection by bovine hypesvirus type 1 and suid herpesvirus type 1, hence, concluded that it possess antiviral activity. In addition, in the study of the effect of carrageenan on lipid metabolism and antioxidant status, Gomez-Ordoriez *et al.* (2012) found out that it increases the moisture in ceacum, anticoagulant capacity of plasma, the proportion of acetic acid and propionic acid, improves antioxidant status in caecum and decreases triglycerides and total cholesterol in healthy rats. However, it did not show prebiotic effect and reduce antherogenic index. Raman *et al.* (2015) displayed the possibility of κ-carrageenan as dietary fiber and its anticarcinogenic effect of on human colon cancer. In addition, it also aids to prevent human papillomavirus acquisition *in vivo* and *in vitro* (Rodriguez *et al.,* 2014).

Phlorotannin

Phlorotannin is the only tannin groups present in brown seaweeds (Fung *et al.*, 2013 & Kadam *et al.*, 2013). It has been applied in many fields, include medicines, cosmetics, pesticides, paints, cements and dyes (Kim, Uddin, Hyun, Kim, Suh, & Lee, 2015a). Its molecular size ranges from 126D to 650 kD (Hu *et al.,* 2016). It is a type of hydrophilic polyphenols consist of phloroglucinol (1, 3, 5-trihydroxybenzene) units. Phloroglucinol is comprised of aromatic ring with three hydroxyl groups. They link to each other and also only present in brown seaweeds (Li, Wijesekara, Li & Kim, 2011 & Kim *et al.,* 2015a). Among the orders of brown seaweeds, Fucales have the greatest amount of fucoxanthin (Lopes *et al.,* 2012).

Phlorotannin is highly soluble in water and is biosynthesized in Golgi apparatus through acetate-malonate pathway (Lopes *et al.,* 2012). There are four groups of phlorotannin depends on the linkages, which are eckols with dibenzodioxin linkages, fuhalols and phloroethols with ether linkages, fucols with a phenyl linkage and fucophloroethols with ether and phenyl linkages (Ferreres *et al.,* 2012). The concentration of phlorotannin (1 to 14%) fluctuates depends on environmental condition, for examples, nutrient, salinity, light availability, UV radiation, and herbivore intensity (Koivikko, Loponen, Honkanen & Jormalainen, 2005). They reported that nutrient availability affects negatively on phlorotannin concentration. This is due to the fact that when the nutrient is available, carbon is used for growing purposes instead of secondary metabolite production. Furthermore, its concentration is vary according to location. It is found highest in temperate and tropical Atlantic and lowest in tropical Pacific and Indo-Pacific region (Lopes *et al.,* 2012).

The primary role of phlorotannin is as cell wall components and secondary function as defensive compounds (Gupta *et al.*, 2011). Phlorotannin can be further subdivided into two groups, soluble phlorotannins and insoluble phlorotannins (Koivikko *et al.*, 2005). They reported that most of the phlorotannin presents as soluble phlorotannin in the cytoplasm and only a minimal amount of insoluble phlorotannin is found in the cell wall of brown seaweed. Soluble phlorotannins are stored in highly refractive, colorless, round or elliptical, and motile vesicles called physodes, in cytoplasm. They play an important role in defensive action on biotic stress condition due to the protein precipitation capacity to prevent from being consumed by herbivores (Lopes *et al.,* 2012). Besides that, it also provides protection against abiotic stress conditions such as UV radiation and oxidation. Insoluble phlorotannins are formed when soluble phlorotannins complex with alginic acid after physodes fuse with cell wall and release their contents (Koivikko *et al.*, 2005). They are part of the cell wall as insoluble phlorotannins and hence, the cell wall needs to be broken down to release phlorotannins from brown seaweeds.

Li *et al.* (2011) stated that phlorotannins has antioxidant, antiallergic, radioprotective, anticancer, anti-human immunodeficiency virus, bactericidal, and enzyme inhibitory effect. Lopes *et al.* (2012) evaluated the biological properties of phlorotannins from ten species of brown seaweed by examining the inhibition effect on nitric oxide generation by lipopolysaccharide-stimulated RAW 264.7 macrophage cells. They also tested the effectiveness of phlorotannins against the common bacteria and fungi found in food contamination. By doing so, they concluded that phlorotannins possess antibacterial, antifungal, and anti-inflammatory activity. In addition, Ferreres *et al.* (2012) proved that phlorotannins have antioxidant and anti hyaluronidase activity. Furthermore, phlorotannins possess anti-obesity property by inhibiting pancreatic lipase to prevent fat absorption and obstruct adipocyte differentiation (Hu *et al.,* 2016). Apart from that, phloroglucinols present in the flowers of *C.citrinus* also found to possess antioxidant, antinociceptive and anti-inflammatory activities (Radulovic, Randjelovic, Stojanovic, Cakic, Bogdanovic & Zivanovic, 2015). Last but not least, Kim *et al.* (2015a) stated the anticancer property of phloroglucinol especially breast cancer as it can target breast cancer stem cells and prevent the disease from relapsing.

Fucoxanthin

Fucoxanthin is the major source of carotenoids, which contributed to 10% of total natural carotenoid production (Bharathiraja, Randjelovic, Stojanovic, Cakic, Bogdanovic & Zivanovic, 2013). Fucoxanthin is an orange colored pigment located in the chloroplast of brown algae (Renny Indrawati, Helen Sukowijoyo, Indriatmoko, Retno Dumilah, Esti Wijayanti & Leenawaty Limantara, 2014). It establishes the brown or olive green color in brown seaweeds (Wu *et al.,* 2014). It has allenic bond, an epoxide and a conjugated carbonyl group in the polyene chain of the molecule and contains L-fucose, sulfate and minor amount of sugars. Its molecular structure is similar to neoxanthin, dinoxanthin and peridin but different to other carotenoids, for example, astaxanthin and β-carotene (Bharathiraja *et al.,* 2013). It is present in brown seaweeds that grow in environments with high intensity of light and oxygen, which cause the generation of free radicals and other strong oxidizing agents.

The composition of fucoxanthin varies among different types of species, from 0.04mg/g to 7.4mg/g of sample (Wu *et al.,* 2014). Terasaki *et al.* (2016) reported that fucoxanthin content shows seasonal and spatial variation. Fung *et al.* (2013) & Terasaki *et al.* (2016) discovered that fucoxanthin content is higher during winter and spring than summer and concluded that the low light and low temperature during winter increase the production of fucoxanthin. Besides that, their study also revealed that the variation in content of fucoxanthin depends on maturation period, temperature, light, nutritional profile of water, geographic location, harvesting time, pre-treatment or handling conditions, and depth (Billakanti, Catchpole, Fenton, Mitchell & Mackenzie, 2013).

Fucoxanthin is pH sensitive. The absorbance range of fucoxanthin is between 420-470 nm. Wu *et al.* (2014) stated that it is stable at pH between 5 and 7 and the stability decreases in high acid or high alkaline solution. They explained that this is due to pigment photodegradation as a result of trans-cis isomerization of fucoxanthin. Hence, it is necessary to store or process fucoxanthin in the dark to prevent loss. Furthermore, fucoxanthin is also heat sensitive. Wu *et al.* (2014) reported that 20% of fucoxanthin is lost when it is stored at 50°C compared to at 25°C. At the same time, they also found that there is no significant difference in fucoxanthin content that is stored at 4°C and 25°C. This indicates that at high temperature, the double bond in fucoxanthin is broken and fucoxanthin is destroyed, but this is not happening at low temperature. Billakanti *et al.* (2013) recommended that do not expose fucoxanthin for long periods of time at temperature more than 50°C due to the possibility of degradation. In addition, they also discovered that the content of fucoxanthin in freeze dried form is lower than that in wet form. They related this condition to the thermal sensitive property of fucoxanthin and concluded that the drying process had degraded it. Furthermore, fucoxanthin is lipid soluble and the common solvent used in laboratory scale are toluene, hexane, acetone, methanol and ethanol.

The benefits of fucoxanthin were highlighted in the reviews by Renny Indrawati *et al.* (2014) and Peng, Yuan, Wu & Wang (2011), which include anti inflammation, antidiabetic, antiangiogenic, antimalarial, antiobesity, antioxidant, chemopreventive, and chemotherapeutic properties. Heo *et al.* (2012) suggested the potential of

fucoxanthin as an anti-inflammatory agent and modulator of macrophage activation based on their findings. Muradian, VAiserman, Min & Fraifeld (2015) examined the effect of fucoxanthin on rodents and concluded that it can prevent and treat obesity, type 2 diabetes, metabolic syndrome and heart disease. They showed that the consumption of fucoxanthin led to redistribution of plasma lipid with increasing high density lipoprotein to low density lipoprotein ratio, decreasing triglycerides content but had no effect on plasma cholesterol level. Moreover, Hu *et al.* (2016) also stated that fucoxanthin inhibits lipid absorption and pancreatic lipase activity, which contribute to the anti-obesity property of fucoxanthin. Kim *et al.* (2013) evaluated the effect of fucoxanthin *in vivo* in mice and shown that the growth of tumor mass in B16F10 cells implanted mice had significantly inhibited, which shown that fucoxanthin has anti-tumor property. Besides that, Bharathiraja *et al.* (2013) studied the effect of fucoxanthin on nephrotoxicity and found that it reduced the cadmium-induced oxidative renal dysfunction in rats; hence, they suggested that it is able to prevent oxidative renal dysfunction from cadmium exposure. In addition, fucoxanthin was proposed as a possible treatment for Alzheimer's disease as it acted as noncompetitive inhibitor to β-site amyloid precursor protein cleaving enzyme 1 that onset the Alzheimer's disease (Jung, Md Yousof Ali, Choi, Jeong, Chung & Choi, 2016). Furthermore, Fung *et al* (2013) studied the antioxidant property of fucoxanthin extracted from brown seaweeds and discovered that it has stronger scavenging activity and reducing capacity than the commercial fucoxanthin. A study done by Kim et al., (2010a) Fucoxanthin induced apoptosis in human leukemia HL-60 cells Kim *et al.* (2010a) revealed that fucoxanthin had increased the generation of reactive oxygen species to prevent the growth of human leukemia HL-60 cells and cause the apoptosis of HL-cells. Thus, they proposed the utilization of fucoxanthin in leukemia treatment. Moreover, Nishikawa, Hosokawa & Miyashita (2012) reported that fucoxanthin had suppressed hyperglycemia and hyperinsulinemia in diabetic or obese mice after two weeks of treatment with fucoxanthin. Hosokawa *et al.* (2010) found out that fucoxanthin reduced the mRNA expression of inflammatory adipocytes involved in insulin resistance on diabetic/obese mice. However, it did not affect lean mice. Additionally, fucoxanthin was proved to have anti-inflammatory property as it decreased the pro-inflammatory mediators in murine macrophage cell RAW 264.7 (Kim *et al.,* 2010b). Last but not least, Kang *et al.* (2014) showed the ability of fucoxanthin in preventing the oxidative damage in cell and organ by hyperglycemia as high glucose level increases the reactive oxygen species generation, lipid peroxidation and cell death.

Diterpene

There are three types of carbon skeletons in diterpenes, known as xenicanes, "extended sesquiterpenes" and dolabellanes. The chemical structure of diterpene is different according to species. For instance, it exists as isopachydictyolal in *D. dichtoma,* dictyol B acetate, pachydictyol A and isopachydictyol A in *D. caribeae,* and 4α-acetyledictyodial in *D.linearis* (Siamopoulou *et al.,* 2004). In seaweeds, it acts as a defensive compound against epibionts, pathogens and herbivores. Hence, the composition of diterpenes is also affected by geographic location and herbivore pressure (Simas, Kaiser, Gestinari, Duarte, Paula & Soares, 2014).

Diterpene exhibit a wide range of bioactivity including cytotoxic, antibacterial, ichthyotoxic and anti-feedant activities. Siamopoulou *et al.* (2004) showed that when the dose used is higher than their maximal non-toxic dose, they are found to possess antiviral activity against *Poliomyelitis virus I* and *Herpes simplex virus I.* They also proved the toxicity of diterpene towards Vero cells. Furthermore, it can also be used as antiophidian agent for the treatment of envenomation by snake and others as it demonstrates antihemolytic, antihemorrhagic and anticoagulant properties and inhibits the important enzymes in *L. muta* crude venom (Moura, Sanchez, Bianco, Pereira, Teixeirra & Fuly, 2011).

Fatty acids

Fatty acids present in seaweeds also demonstrate bioactivity on human health. Seaweeds have a rich source of 20 carbon atom polyunsaturated fatty acids (PUFA) (Pal, Kamthaniam & Kumar, 2014). According to the metabolic connections, PUFA are normally grouped into two groups, which are linoleic acid (*n*-6 fatty acids) and α-linolenic acid (*n*-3 fatty acid) (Holdt & Kraan, 2011). Long chain PUFA presents in large amounts in seaweeds, majorly of them are *n*-3 fatty acids and monounsaturated fatty acids. Among the PUFA, the two major fatty acids present in seaweeds are docosahexanoic (DHA; 22:6 *n*-3) and eicosataenoic (EPA; 20:5 n-3). Prostaglandin and gracilariales are the products from metabolism of these fatty acids through oxidative pathways. Furthermore, eicosapentaenoic acid (EPA) is a 20 carbon chain polyunsaturated acid with the last double bond at the third carbon from methyl terminus or five double bonds at carboxyl terminus. In seaweed, it exists as a complex lipid molecule through esterification (Pal *et al.*, 2014). Besides, seaweeds have fatty acids that are not present in other organisms, which are *n*-3 PUFA 18:4 (*n*-3). The composition of fatty acids in seaweeds also varies according to species. Generally, green seaweed has 4.9% to 23.1% of 16:4 fatty acids in total fatty acids with high amount of 16:0, 18:1 and 18:3 acids. Brown seaweed has unsaturated fatty acids predominate in it while red seaweed has saturated acid predominate. Both Phaedopyceae and Rhhodopyceae have same amount of *n*-6 and *n*-3 acids.

Obesity and cardiovascular disease can be prevented by better understanding of the biological use of fatty acid in body. Besides, normal cell function had proved to be

related to fatty acid with at least two methylene side chains. In addition, polyunsaturated fatty acids are imperative in membrane fluidity regulation, tissue and cellular metabolism, thermal adaptation and oxygen and electron transport. It is also crucial in development and physiology modulation. Furthermore, it acts as a precursor for eicosanoids and hormones, for instances, leucotriens, prostaglandins and thomboxanes. Polyunsaturated fatty acids were found to exhibit anti-inflammatory activity (Pal *et al.*, 2014). Holdt *et al.* (2011) stated that *n*-3 polyunsaturated fatty acids exhibit anti-inflammation, anti-hypertension, anti-artheriosclerosis and immunoregulation effects.

Sterols

Sterols are important nutrients present in seaweeds. Different species of seaweeds have different types of sterol. Phaedophyta contains cholesterol and brassicasterol while chlorophyta contains ucocholesterol, methylene cholesterol, β-sitosterol, fucosterol and cholesterol. Rhodophyta contains sitosterol, chalinasterol, cholesterol, demosterol and fucosterol.

Fucosterol and β-sitosterol were found to decrease the cholesterol level in human and animal serum. Furthermore, cholesterol affects cell membrane fluidity, acts as a secondary messenger in developmental signaling, and as a precursor to steroids hormones and fat soluble vitamins. (Pal, Kamthaniam & Kumar, 2014). It was found to exhibit antibacterial property by disrupting the intra and inter cellular cell-cell communicaton as it can prevent furanone on the quorum sensing mechanism. The same finding is reported by Kavita, Singh & Jha (2014), they found that the antibacterial properties of red seaweed on Gram-positive and Gram-negative bacteria are due to a cholesterol derivative, 24-propylidenecholest-5-en-3β-ol. They also proposed that it can be used as antimicrobial compounds in pharmaceutical industries.

Agar

Agar is structural polysaccharide present in red seaweed (Ramnani *et al.,* 2012). It is a sulphated polysaccharide with similar functional properties as carrageenan, comprises of agro pectin and agarose. This galactan has β-9(1-3)-D-galactose and α (1-)-3,6-anhydro-L-galactose residues linked by α (1-3) and β (1-4) alternate bonds and esterified by a little amount of sulphatein (6% w/w) (Holdt *et al.*, 2011 & Ramnani *et al.,* 2012). It can be methylated, pyruvated or sulfated. The quality and its composition vary due to different environmental condition, physicochemical property, reproductive cycle and growth. Agar is widely applied in medical, food and pharmaceutical industry and as biological culture media (Holdt *et al.*, 2011).

In pharmacology study, it can affect ultraviolet ray absorption, exhibit anti-aggregation effect on red blood cell and lower the blood glucose level. Furthermore, it also shows anti-tumor and antioxidant properties, suppresses the enzyme associated with the production of nitric oxide and decreases pro-inflammatory cyctokine production. In addition, it is also able to induce limit on cell apoptosis *in vitro,* which enables it to be used in cancer treatment (Holdt *et al.*, 2011). Besides, Ramnani *et al.* (2012) found that gut bacteria ferments some low molecular weight polysaccharides derived from agar which isolated from seaweeds and hence, proposed the possibility of them to be used as prebiotics. These complex polysaccharides resist enzyme degradation and undergo microbial fermentation in gut.

Ulvan

Ulvan is highly charged, water soluble sulphated polysaccharides present in cell wall of green seaweeds (Patel, 2012). It has molecular weight ranges from 189 to 8200 kDa and made up approximately 8-29% of the seaweed dry weight. In seaweed, it functions as a plant defense elicitor to inhibit pathogenic fungus *Colletotrichum trifolii* infection (Jaulneau *et. al.,* 2010). It has complex and vary chemical structure It is constituted of disaccharides such as iduronic acid, aldobuironic acid, (1→4)-D-glucuronic acid-(1→4)-L-rhamnose-3-sulphate-(1→) and monosaccharides such as xylose, uronic acid and rhamnose. Water soluble ulvan and insoluble cellulose-like material are two types of ulvan present in seaweeds (Holdt *et al,* 2011).

Patel, S. (2012) listed the health benefits of ulvan to human, which are antiviral, antitumour, immunostimulatory, anticoagulant or antithrombotic, antioxidant, hyperplasia prevention, anti-inflammatory, lipid lowering, antibacterial, pulmonary fibrosis, antiprotozoan, gastrointestinal, regenerative and nano medicine applications. Jiao, Yu, Zhang & Ewart (2011) reported that ulvan demonstrated anticoagulant activity as it inhibits thrombin and modulate heparin cofactor which prolong the clotting time. Besides, they also stated that high sulfate content ulvan has improved antioxidant activity. The antioxidant activity of ulvan depends on the molecular weight of ulvan, stronger antioxidant activity is demonstrated in low molecular weight ulvan compared to larger fractions (Cunha & Grenha, 2016). Ulvan also possesses antilipidemic effect as it normalizes the hypertriglyceridemia and raises high density lipoprotein cholesterol in rat fed with high cholesterol diet (Jiao *et al.,* 2010). Cunha *et al.* (2016) reported the similar findings, which ulvan increase the high density lipoprotein cholesterol while decreasing low density lipoprotein cholesterol, total serum cholesterol and triglycerides. Antilipidemic effect of ulvan also depends on the molecular weight, whereby low molecular fraction shows higher effectiveness on high density lipoprotein cholesterol and total serum while high molecular fraction is more effective on high density lipoprotein cholesterol (Cunha *et al*, 2016). Ulvan inhibit abnormality induced by D-galactosamine in ulvan pretreated rat, which shows

lipid lowering property (Jiao *et al.,* 2010). Holdt *et al.* (2011) stated that ulvan was found to exhibit anti-influenza properties and able to modify the proliferation, modify the expression of surface glycosyl markers and transforming growth factors, and modify the adhesion of tumoral and normal human colonic cells. Furthermore, it also shows inhibition against herpes simplex virus (Cunha *et al.,* 2016). Besides, iduronic, synthesis from sugar in ulvan can be used to inhibit thrombosis. In addition, gastric ulcer can be treated by using rhamnose, rhamnan and oligomers from ulvan.

Bioactive protein

Protein made up approximately 10% to 44% dry weight of seaweed (Holdt *et al,* 2011). Normally, all essential amino acids are found in seaweeds. The protein content in seaweed varied according to location, species, bioactic interactions and temporal and spatial changes in the environment parameters (Yu, Jantan, Ahmad & Wong, 2014). Seaweed is rich in aspartic acid, acidic amino acid and glutamic acid while threonine, sulphur amino acids (methionine and cysteine), tryptophan, threonine, histidine, lysine are limited amino acids in seaweeds but their level are still higher than those present in terrestrial plants. Protein content in seaweeds varies according to species. Brown seaweed has rich source valine, glycine, leucine, lysine, alanine, tyrosine, methionine, cysteine, histidine, tryptophan; while Rhodophyceae contains acidic amino acids, aspartic acid and glutamic acid, proline and arginine. Besides, bioactive lectin is also found in seaweed. It is a carbohydrate bound protein present in green macroalgae (Holdt *et al,* 2011).

Lectin has a wide range of biological processes such as cancer metastasis and differentiation, apoptosis induction, cell-cell communication, host-pathogen interactions. Furthermore, it also possesses antibiotic, anti-adhesion, anti-HIV, mitogenic, cytotoxic against colon cancer and cervical cancer, anti-bacterial, anti-nociceptive, anti-inflammatory, increases red blood cells agglutination and disease related alterations of glycan synthesis, such as infectious agents like viruses, parasites, fungi and bacteria. Agglutinin glycoprotein has mitogenic effect on lymphocytes, cyctotoxic and anticancer activity while mycin-binding agglutinin has anti-inflammatory activity. Furthermore, amino acids such as taurine, laminine and kanoids have anti-hypertensive, anti-diabetic, antioxidant, preventive effect of vascular diseases and chronic hepatitis, insecticidal, anthelmintic, neuroexcitatory and depress smooth muscles contraction. Peptides such as depsipeptide (Kalahide F), oligopeptide, hexapeptide (SECMA 1) and cyclic pentapeptid (Galaxamide) have anti-inflammatory, anti-cancer, anti-tumor, anti-AIDS, mitogenic, antioxidant and anti-angiotensin, and blood pressure, cholesterol and glucose level reduction (Holdt *et al,* 2011). Besides, Yu *et al.* (2014) reported that kahalalides, sequence of amino and hydroxyl carboxylic acid residues, exhibit anticancer property against prostate cancer.

Carotenoids

Carotenoids are compounds synthesized in photosynthetic organisms. It is tetraterpenoids which has C_{40} molecular backbone with 11 conjugated double bonds. As marine photosynthetic organisms, seaweeds contain carotenoids which act as basis for the green, brown and red seaweed classification (Mikami & Hosokawa, 2013).

In seaweed, there are green, lipid soluble pigments known as chlorophyll present in it to carry out photosynthesis process (Mikami *et al.,* 2013). Before ingestion by human, through food processing, it is converted into pheophorbide, pyropheophytin and pheophytin. These derivatives have bioactivity such as anti-mutagenic property. The β-carotene has provitamin A and antioxidant activity, prevent cancer and reduce cardiovascular disease. Astaxanthin has ten-fold higher antioxidant activity compared to other carotenoids. Zeaxanthin and lutein are able to reduce the risk of opthalmological disease. Tocopherol has antioxidant, anti-inflammatory, antiviral, anti-tumour, hepoatoprotective, lipase inhibition, hypocholesterolemic, neuroprotectivem, liver protecting activity and can be used for atherosclerosis treatment (Holdt *et al,* 2011).

Conclusions

In short, seaweed contains a lot of bioactive compounds that have health benefits to human. They possess a wide ranges of bioactivities include anti-inflammatory, anti-coagulant, anti-oxidant, anti-viral, anti-tumor and others. As seaweeds are largely available marine organisms, it has a big potential to replace present chemical synthesized drugs.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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