1. INTRODUCTION

Seaweeds are among the oldest members of the plant kingdom. They have little tissue differentiation, no true vascular tissue, no roots, stems or leaves and flowers (Dhargalkar and Pereira, 2005). Seaweed or sea vegetables are rich in polysaccharides, vitamins, minerals, bioactive substances like polyphenols, proteins, lipids and carotenoid that possess antioxidant, antibacterial, antiviral and other beneficial functions. Marine products are currently of considerable interest in the food and pharmaceutical industries for the development of antioxidants (Ngo et al., 2010).

Seaweeds greatly influence environmental conditions for other types of marine life by providing food, shade protection from waves and as substrate for attachment of other organisms. Seaweeds were one of the first groups of marine organisms whose natural product chemistry was studied extensively because of their abundance in shallow waters. During the past 30 years, marine natural product chemists have reported the discoveries of a large number of novel metabolites with useful pharmacological properties (Faulkner, 2000). The majority of the studies on seaweeds occurred after the development of many of the useful mechanism bioassays used today.

Seaweeds are harvested for different purposes and utilised differently around the globe. In the Pacific and Asian cultures, seaweed has long been incorporated into human diets such as “nori” (Porphyra sp.) as sushi wrappings; “hijiki” (Hizikia fusiforme (Harvey) Okumaru), “kombu” (Laminaria sp.), “wakame” (Undaria pinnatifida (Harvey) Sawingar) that are consumed in soups, salads and vegetable dishes, and “Limu Palahalaha (Ulva fasciata) in Hawaiian snacks. Besides these traditionally uses, seaweeds are also incorporated in modern commercial food products in Asian markets, most commonly in
confectionery products (Foodnavigator, 2006). In western countries, seaweeds are mainly
developed as a hydrocolloid used in processed food in the form of additives (from E400
to E407), or for pharmaceutical industry purposes as a tablet encapsulation agent.
However, with the increase of Asian cuisine consumption in western countries together
with the known health benefits of seaweed consumption; other cultures have started to
include seaweed into their diets.

Garibaldi et al. (2010) reported that, brown seaweed production remained the
highest among cultured seaweeds; Laminaria japonica (4.8 million tonnes) followed by
Undaria pinnatifida (1.8 million tonnes). The second highest production of cultured
seaweeds are red seaweeds; Eucheuma sp., seaweeds (Kappaphycus alvarezii and
Eucheuma sp., 3.8 million tonnes), Porphyra sp., (1.4 million tonnes) and Gracilaria sp.,
(1.4 million tonnes). It is apparent that the demand for brown seaweeds remained high
and profitable over the past 20 years (1991-2008) and would most likely continue to do
so in the following years.

Several studies on different seaweed species have shown that brown seaweeds
have a higher antioxidant capacity than red or green seaweed (Jiménez-Escrig et al.,
2001; Matanjun et al., 2008; Prabhasankar et al. 2009). Phlorotannins are the only group
of tannins present in brown seaweed. These extracts have shown protective effects
against hydrogen peroxide-induced cell damage by acting as free radical scavengers
(Tierney et al. 2010), reducing agents and metal chelators (Ngo et al., 2010;
Tierney et al., 2010). On the contrary, fucoxanthin is the dominant carotenoid in brown
seaweeds. Although less attention has been paid to the physiological effects of carotenoid
in seaweeds, fucoxanthin has recently attracted much attention due to its strong
antioxidant properties that showed significant anti-cancer, anti-obesity and anti-inflammatory effects (Miyashita and Hosokawa, 2008).

Seaweeds contain antioxidant substances of very different nature that may either be water soluble or lipid soluble compounds. Water soluble antioxidants such as polyphenols, phycobiliproteins and vitamins (vitamin C) react with oxidants in the cell cytosol and blood plasma as an excellent free radical scavenger (de Quiros et al., 2010; Sies, 1997). On the contrary, lipid soluble compounds such as carotenoids and tocopherols can act as free radical scavenger and singlet oxygen quenchers (Airan thi et al., 2011; Sachindra et al., 2007) that protect cell membranes from lipid peroxidation. Brown seaweed species examined in several experiments has been reported to have a higher antioxidant capacity than red or green seaweed (Jiménez-Escríg et al., 2001; Matan jun et al., 2008; Prabhasankar et al., 2009).

Polyphenols in general are categorized into distinct groups according to their structures by the presence of several hydroxyl groups on aromatic rings, such as the flavonoids, phenolic acids, stilbenes and lignans (Tierney et al., 2010). They are widely found in the plant kingdom as secondary metabolites responsible for pigmentation, reproduction, growth and has mechanism defense against pathogens (Yuan, 2007; Yvonne, 2007). Polyphenols have demonstrated multifunctional antioxidant activity, due to their phenol rings acting as electron traps to scavenge peroxyl, superoxide anions and hydroxyl radicals.

Phlorotannins are the only group of tannins present in brown seaweed. The brown to black coloration of Phaeophyceae results from phlorotannins and their oxidation products. They are reported to be highly hydrophilic (Yvonne, 2007; Burtin, 2003; Yuan,
2007) and are also suggested to be responsible for the higher antioxidant capacities of brown seaweeds (Airanthi et al., 2011). These extracts have shown protective effects against hydrogen peroxide-induced cell damage by acting as free radical scavengers (Tierney et al., 2010), reducing agents and metal chelators (Ngo et al., 2010; Tierney et al., 2010).

The recognition of important bioactive molecules of pigments in seaweed lipids has long been acknowledged (Huang and Wang, 2004; Yuan, 2007). Chlorophylls are the major photosynthetic pigment whereas carotenoids are the secondary photosynthetic pigment. Although both classes of pigments have antioxidant activity, it was the compounds in carotenoids which have strong biological effects to prevent disease (Sachindra et al., 2007). Most carotenoids are polyunsaturated hydrocarbons containing 40 carbon atoms and two terminal ring systems. Those carotenoids which are composed entirely of carbon and hydrogen are known as carotenes, whereas those that also contain oxygen are termed xanthophyll (Roh et al., 2008).

The carotenoid extracted from brown seaweeds is predominately rich in fucoxanthin followed by violoxanthin as the second major xanthophyll and β-carotene, probably the single carotene (Burtin, 2003; Haugan and Liaaen-Jensen, 1994). The strong antioxidative properties of β-carotene in fruits and vegetables have been well established. However relatively less attention had been paid to the physiological effects of carotenoid in seaweeds. Fucoxanthin on the other hand had recently attracted much attention due to its strong antioxidant properties that show significant anti-cancer, anti-obesity and anti-inflammation effects (Miyashita and Hosokawa, 2008).
Tocopherol is another lipophilic compound that had strong antioxidant activity. It has been extracted from several brown seaweeds (Fucus vesiculosus, Fucus serratus, Hijikia fusiformis and Laminaria digitata), with δ-tocopherol making up the majority; with γ-tocopherol and traces of α-tocopherol also found (Le Tutour et al., 1998).

Fucoxanthin is an abundant marine xanthophyll that contains an allelic bond and two epoxy groups. It is estimated to account for more than 10% of total carotenoid produced in nature (Miyashita and Hosokawa, 2008; Nakazawa et al., 2009; Terasaki et al., 2009). This characteristic lipid component of brown seaweeds is bound to several proteins, together with chlorophyll ‘a’, to form fucox-Chl a-protein complexes in the thylakoid, where it acts as a light harvesting and energy transferring pigment (Kim et al., 2011). Fucoxanthin in particular has been extensively investigated with respect to its strong antioxidant activity.

Yan et al. (1999) demonstrated that the major active compound isolated from the carotenoid extract in Hijikia fusiformis was fucoxanthin that showed strong DPPH radical scavenging activity. The electron spin resonance method employed to investigate the quenching ability of fucoxanthin against the organic radicals DPPH, radical adduct of nitrobenzene with linoleic acids (NB-L) and 12-doxy-steric acid (12-DS) indicated that in the presence of fucoxanthin, the ESR signals for these radicals are significantly decreased by 28%, 57%, and 66% respectively (Sachindra et al., 2007). From the structural point of view, it is suggested that the presence of the unique double allenic carbon (c-7, 201.84ppm) and two hydroxyl groups in fucoxanthin confer additional stability and resonance stabilisation within the conjugated double bond structure are responsible for the higher antioxidant activities (Sachindra et al., 2007; Yan et al., 1999;
Yuan, 2007). Although fucoxanthin is known for its strong antioxidant activities, investigations on its involvement in the antioxidant system are limited and vague (Airanthi et al., 2011).

Fucoxanthin in its pure form is vulnerable to oxidation. Nonetheless it is fairly stable in the presence of co-existing antioxidants such as polyphenol. Fucoxanthin identified in the dried form of algae stored at ambient temperature (Miyashita and Hosokawa, 2008) is present in lower amounts indicating that the process of drying could decomposed fucoxanthin. The content of fucoxanthin was also reported to vary significantly with season and the life cycle of the algae, peaking between the winter and spring (mature phase of sporophyte) and lowest during summer (senescence phase) (Terasaki et al., 2009).

An antioxidant can be defined as any substance, that when present at low concentrations compared with those of an oxidisable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1993). Compounds such as polyphenols, carotenoids and fucoidan are currently extracted from algae for commercial purposes. The technology also exists to commercially extract antioxidants such as vitamin E from algae however most antioxidant compounds are extracted from other sources (vitamin E from edible oils) or industrially synthesized (vitamin C). The predominant antioxidants in seaweeds are in the categories of pigments, polyphenols and fucoidan.

Seaweeds contain a diverse range of carotenoids unlike higher plants which contain a uniform profile of a small number of carotenoids (Demmig-Adams et al., 1996). The characteristic colour of brown algae is due to the dominant xanthophyll, fucoxanthin, which masks other pigments including chlorophylls and carotenoids. The
dominance of the phycobiliprotein pigments, phycoerythrin and phycocyanin give red algae their distinctive colour. The dominance of chlorophyll ‘a’ and ‘b’ gives green seaweed its colour. In plants, carotenoids serve two primary functions, in photosynthesis, to collect light and pass it to chlorophyll and to protect against light induced damage to chlorophyll and other organelles. Chlorophyll ‘a’ absorbs the majority of light (in green and brown algae) and is assisted by other chlorophylls ‘b’, ‘c’, ‘d’ and ‘f’ and pigments such as fucoxanthin, siphonaxanthin, and peridinin, which absorb light at different wavelengths to that of chlorophyll ‘a’. Phycobiliproteins are the main light-absorbing pigments in red algae absorbing light in the range 520 nm to 630 nm which allows red algae to inhabit deep water. Absorbed light is subsequently used in the photosynthesis process to produce energy and other components. Carotenoids such as violaxanthin, antheraxanthin and zeaxanthin also protect seaweed by dissipating potentially harmful photo energy via the xanthophylls cycle (Demming-Adams et al., 1996).

Due to the unpredictable nature of the marine environment, seaweed is subjected to a number of abiotic stresses. Seaweeds have developed a wide range of mechanisms to protect against these external stresses. A study by Collén et al. (2007) identified a number of antioxidant proteins and enzymes upregulated in Chondrus crispus in response to abiotic stress including ascorbate peroxidase, catalase (CAT), dehydroascorbate reductase, glutathione peroxidase (GPx), glutaredoxin glutathione reductase, superoxide dismutase (SOD), thioredoxin, cytochrome P450s, glutathione S-transferases, xenobiotic reductase and many other proteins. The concentration of the antioxidants in algae are dependent on a range of environmental factors such as salinity, water and air temperature, UV-light, nutrient availability, maturity and exposure to heavy metals. Genetics may also
play a role in the phlorotannin content of seaweed (Jormalainen et al., 2003; Jormalainen and Honkanen, 2004).

The majority of antioxidants are tightly bound within the seaweed matrix; for example fucoidan and a significant amount of phlorotannins are tightly bound within the cell wall, while carotenoids are membrane-bound in the chloroplasts and chromoplasts. Therefore, these components must be extracted before their levels can be properly quantified and before they can be utilized as food ingredients or for other applications. A wide range of both simple and advanced techniques for the extraction of antioxidant compounds from algae have been developed. The most commonly utilised type of extraction involves the use of solvent. The exact solvent extraction technique employed depends on the solubility of the desired compound, for example polar compounds like phlorotannins solubilise very easily in highly polar solvents such as water, acetone and alcohols. In contrast, nonpolar or lipophilic compounds such as vitamin E and carotenoids are insoluble in water, and can only be extracted using low or non-polar solvents like hexane and chloroform. Another factor governing the extraction technique depends on the desired purity of the compound.

Three major classes of photosynthetic pigments found in algae are chlorophylls, carotenoids (carotenes and xanthophylls), and phycobilins. Seaweeds are classified into three major groups according to their photosynthetic pigments which are green, brown and red algae.

A range of chromatography-based fractionation techniques have been used to isolate pure compounds from crude seaweed extracts prepared using solvent and enzyme extraction methods. Fucoxanthin has been extracted from Sargassum siliquastrum using a
combination of solvent extraction, silica column, chromatography, Sephadex chromatography and HPLC (Heo and Jeon, 2009a).

Fucoxanthin is a major marine carotenoid found in brown seaweed (Chandini et al., 2008b). It has a unique structure, which contains an allenic bond and a 5,6-epoxide in its molecule. Some examples of familiar brown seaweed include Sargassum sp., Undaria sp., Hizikia sp., Laminaria sp., and Cladosiphon sp. Fucoxanthin exhibits anti-carcinogenic, anti-obesity, anti-inflammatory, anti-angiogenic, and antioxidative activities (Das et al., 2005; Heo et al., 2008; Kotake-Nara et al., 2001; Maeda et al., 2006; Okuzumi et al., 1990; Shiratori et al., 2005; Sugawara et al., 2006). Orally administered fucoxanthin is safe in terms of mutagenic potential (Beppu et al., 2009). Despite these desirable features, the utilization of fucoxanthin of a high purity in functional food preparations has been restricted because of the instability of fucoxanthin against oxidation and heat during processing. Nonetheless, although pure fucoxanthin is unstable, the fucoxanthin extracted from the alga is rendered stable by the coexisting antioxidants such as polyphenols. Therefore, the optimal conditions for the production of Cladosiphon okamuranus powders enriched with stable fucoxanthin were tested and determined. The obtained powders would also have additional health benefits derived from the coexisting fucoidan, fucosterol, fibers, minerals, and antioxidants.

In addition, Yan et al., (1999) demonstrated that carotenoids have a strong radical scavenging activity and are found as a major antioxidant in marine algae (Nomura et al., 1997). Young and Lowe, (2001) indicated that structure, physical form, location or site of action, potential interaction with another antioxidant, concentration and partial pressure to oxygen may affect the antioxidant activities of carotenoids in biological systems.
Fucoxanthin obtained from *Padina tetrastromatica* has shown higher potential to be used as an antioxidant than β-carotene in modulating antioxidant enzyme in plasma and liver of retinol deficient rats (Sangeetha *et al.*, 2009; Ravi Kumar *et al.*, 2008). However, the exact mechanisms of action how fucoxanthin exerts antioxidative effect in rat induced by retinol deficiency are not yet completely understood. Moreover, the cytoprotective effect of fucoxanthin against ROS formation induced by H₂O₂ in monkey kidney fibroblast (Vero) cells has been observed (Heo *et al.*, 2008). Two hydroxyl groups present in the ring structure of fucoxanthin may correlate to the inhibition of ROS formation. Indeed, it has been reported that the number of hydroxyl groups on the ring structure is correlated with the effects of ROS suppression. Moreover, it has also been shown that some marine algal sulfated polysaccharides (SPs) can be used as potent antioxidants (Wijesekara *et al.*, 2010; Jiao *et al.*, 2011). Antioxidant activity of marine algal SPs depends on their structural features such as degree of sulfating, molecular weight, type of the major sugar and glycosidic branching (Qi *et al.*, 2005; Zhang *et al.*, 2003). However, bioactivities of marine algal carotenoids and SPs against oxidative stress in the CNS have not been demonstrated yet.

The wide diversity of seaweeds and numerous undiscovered unique metabolites present in seaweeds are interesting sources to increase numbers of novel drugs against neurodegenerative diseases. The wide range of biological activities associated with natural compounds derived from seaweeds such as phlorotannins, alginates, fucoidan, sarguquinoic acid, SPs and carotenoids increase the potential to expand the neuroprotective effects and health beneficial value of seaweeds in the pharmaceutical
industry. Until now, most of the biological and neuroprotective activities of seaweed and its natural compounds have been observed in vitro or in mouse model systems.