Screening South Australian Native Seaweeds for Antioxidant Activities

By

Navjot Kaur

A Thesis

Submitted to Centre of Marine Bioproducts Development

Flinders University,

In the partial fulfillment of the requirements

For the degree of

Master of Biotechnology Studies

Department of Medical Biotechnology

Faculty of Health Science

Flinders University of South Australia

November, 2016

Table of Contents

List of Tables

List of Figures

Acknowledgements

First and foremost, I would like to express my deepest gratitude to my supervisor, Professor Wei Zhang for his valuable support, guidance constructive feedbacks, close monitoring for my research and thesis writing. I heartily appreciate and so much thankful to Peng Su, a friendly research assistant for his valuable guidance, instructive advice, continues support throughout the project, through knowledge during the experiment accomplishment and for the training to the use of several instruments. Moreover, Peng su and Dr. Andrew Lorbeer collected seaweeds for this project from Rivoli Bay Beachport, South East coast of Adelaide, South Australia. So, I give special thanks to both of them for their guidance and collection of seaweeds.

My deepest appreciation is conveyed to the Head of the Medical Biotechnology, Professor Chris Franco and Professor Fiona Young for arranging the project and timetable for the Masters student. I would like to thank my mentor Aim who guide me and provide great support in my experiments, calculations and give me valuable suggestions. Special thanks given to Dr. Hanna Krysinska for providing me information regarding chemicals, equipment and safe operating procedures which are quite helpful during work in a lab.

Last but not least, I would like to convey my sincere thanks to my beloved family, especially my very supporting husband, my all friends and all colleagues for their continuous support and encouragement given throughout my project.

Abbreviations

^0C	Degree Celsius
AAPH	2,2-azobis(2-aminopropane) dihydrochloride
ABTS	2,2-azobis(3-ethylbenzothiazoline 6-sulphate)
ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
APS	Almmonium persulphate
AST	Aspartate aminotransferase
BG	Background
BHT	Butylated hydroxytoluene
DAD	Diode array detector
DPHC	Diphlorethohydroxycarmalol
DSW	Dry seaweed weight
DPPH	1,1-diphenyl-2-picryl-hydrazyl
DW	Dry weight of seaweed
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EtOH	Ethanol
FE	$FeSO_4$ equivalents
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalents
H	Hour
HAT	Hydrogen electron transfer
H_2O_2	Hydrogen peroxide
HO_2	Hydroperoxyl
HOCl	Hypochlorous acid
HPLC	High performance liquid chromatography
Min	Minute
$O_2^{\cdot-}$	Superoxide

O_3	Ozone
OH	Hydroxyl
ORAC	Oxygen radical absorbance capacity
PBS	Phosphate buffer saline
PGEs	Phloroglucinol gram equivalents
RO_2	Peroxyl
ROS	Reaction Oxygen species
ROS	Reactive oxygen species
s	Second
SD	Standard deviation
SET	Single electron transfer
SOP	Standard operating procedure
TE	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity
TP	Total phenolic
TPTZ	Tripyridyltriazine
TRAP	Total radical trapping antioxidant potential
UV	Ultraviolet

Declaration

I certify that this thesis does not include any previously submitted material for a degree or diploma in any university, all the content is best of my knowledge and belief and it does not contain any previously published material or written content by another person except few references are made in the text.

Signed.....

Date.....

Abstract

Sea weeds provide an attractive opportunity as a utilization resource due to their fast growth rates comparatively terrestrial plants and wider applications in natural health and cosmeceuticals. South Australia is one of the richest region in marine macroalgal diversity throughout the world which contain approximately 123 species of chlorophyta (green algae), 203 species of Phaeophyta (brown algae) and about 800 species of Rhodophyta (red algae). This study illustrates the information regarding polyphenolic compounds and antioxidant activities of the selected seventeen South Australian native seaweeds. The main aim of the study is to extract polyphenols rich compounds from selected seventeen South Australian native seaweeds to screen antioxidant activities which possess potential applications in health care and cosmeceutical products. The seaweed phlorotannins extracted by 90% ethanol conventional extraction method and yield measured after freeze drying the crude extract at -80^0C. 0.5g of seventeen selected seaweeds dried biomass used for extraction and according to analysis results of total phenolics content (TPC) antioxidant activities by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assay 10g of *Ecklonia radiata* chose for extraction and analysis at large scale to compare the results with *E.radiata* (0.5g). Phlorotannin assay used to determine the total phenolics content and results were expressed in phloroglucinol gram equivalents (PGEs) / 100g DSW (dry seaweed weight). Antioxidant activities of selected seventeen seaweeds were obtained by using FRAP and ORAC assay and results are expressed in mmol $FeSO_4$ equivalents (FE) /100g DSW and µmol trolox equivalents (TE) /100g DSW.

The phlorotannin assay, FRAP assay and ORAC assay were performed with Mili-Q water and 90% ethanol for comparative results to get the accuracy in result data. On the whole, 90% ethanol tested seaweeds in all chemical assays gave high total phenolics content and antioxidant activities comparatively obtained by Mili-Q water. Five genera of seaweeds gave high phenolics content which ranged from 1.01 to 5.4g PGEs /100g DW of seaweed with mili-Q water and 1.7 to 5.5g PGEs /100g DW of seaweed with 90% ethanol. They were *Ecklonia radiata* > *Ecklonia radiata* (10g) > *Sargassum sp.* > *Cystophora monilifera* > *Cystophora platylobium* > *Carpoglossum confluens.* In terms of FRAP antioxidant activities, *[Ecklonia radiata* (28.3 mmol FE /100g DSW), Ecklonia *radiata* (10g) (29.7 mmol FE / 100g DSW) with Mili-Q water] and [*E.radiata* (57.1 mmol FE/ 100g DSW) and for *E.radiata* (10g) gave (48.3 mmol FE /100g DSW) with 90% ethanol], [*Cystophora monilifera* (13.5 mmol FE/ 100g DSW) with MIli-Q water and with 90% ethanol (18.6 mmol FE /100g DSW)] gave high antioxidant activities. On the other hand, *Ecklonia radiata*, *Sargassum sp.* and *C.monilifera* showed high antioxidant activities in ORAC assay with Mili-Q water 623.7 to 794.1 µmol TE/ 100g DSW and with 90% ethanol 80.3 to 820.5 µmol TE /100g DSW. In conclusion, this study successfully identified four South Australian native seaweed species which are best sources of antioxidant activities with the potential of commercial applications of healthcare and cosmeceutical products. Results data indicated that South Australian native seaweeds are potent natural resources in ferric reducing and radical scavenging capacities. These properties are beneficial for the development of nutritional products in pharmaceutical and in cosmeceutical industries.

Chapter 1: Introduction and literature Review

1.1 Introduction

Oxidative stress produced due to imbalance within reaction oxygen species (ROS) and antioxidant defense which cause oxidative damage (Wolfe and Liu, 2007). Reactive oxygen species (ROS) are the species which exhibits both oxygen radicals, such as superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), peroxyl ($RO_2^{\cdot}$), and hydroperoxyl ($HO_2^{\cdot}$) radicals, and various non-radical oxidizing agents, including hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and ozone (O_3), that can be transformed easily to into free radicals. ROS are highly reactive species those formed naturally during oxygen metabolism and scavenged from endogenously produced antioxidants and detoxifying enzymes which give protection against oxidative stress (Ahn et al., 2006).

ROS cause damage to cell organelles such as DNA, cell membranes, proteins, lipids and other cellular constituents which lead to serious human diseases such as atherosclerosis, rheumatoid arthritis, cancer as well as ageing (Heo et al., 2005). Natural immunity of human is not sufficient to combat with elevated ROS levels therefore people try to get antioxidants from food sources such as fruits and vegetables. But these commodities not enough to provide satisfactory antioxidants so natural antioxidant based neutraceuticals, cosmetics and cosmeceutical products required to overcome this problem (Buricova L., 2008).

ROS production increases due to Ultravoilet (UV) irradiation and ageing that cause alteration in function and structure of protein which results impaired function of skin. Therefore, antioxidants required for the treatment of skin ageing and for health purposes. From now, seaweeds are the rich source of potential antioxidant compounds and have been used traditionally to fight against ROS for treatment of skin (anti-ageing products) and for human health (health care products) (Ganesan et al., 2008).

The main aim of the study is to extract polyphenolic rich compounds from selected seventeen South Australian native seaweeds to screen antioxidant activities which possess potential applications in health care and cosmeceutical products.

1.2 Cosmeceuticals

Cosmeceuticals are new hot topic and fast developing sector in the field of cosmetics and in natural health care industry. Cosmeceuticals are the combination of cosmetic and pharmaceutical hybrid ingredients which provide beauty as well as benefit to health. Cosmeceuticals are serving as bridge between cosmetics and pharmaceuticals because they are specifically made for cosmeceutical use as well as medicinally use (Dureja H, 2005). These products contain key ingredients and nutrients which include vitamins, proteins, minerals, botanical extracts, polysaccharides, carotenoids, polyphenols, terpenoids and marine antioxidants to provide healthy skin, nails, hair to cellular level without affecting the structure and function of the cell (Kim, 2014).

Although, many synthetic cosmeceutical products launched in the market but their demand losing due to more beneficial effects of natural cosmeceuticals which contain novel bioactive ingredients from marine resources (Kim, 2012). Due to presence of antioxidative properties in phlorotannins from aquatic brown algae (Phaeophyta) and they are of great attention to manufacturers of nutritional supplements and Cosmetic and cosmeceutical products (Kim, 2014). Marine antioxidants are active compounds of cosmeceuticals which used for the prevention of oxidation of fat and oils. The oxidative rancidity of fats and oils is occurs due to oxygen degradation in air and by using free radicals that happens in chain reactions. The color of the oxidized fat become yellowish and cause undesirable odor which impair the cosmetic (Elena M. Balboa et al., 2015).

According to different mode of action, different types of antioxidants are established such as reducing agents which readily oxidized and avoid the oxidation of active compounds, ascorbic acid and thiourea are the blocking agents that inhibit the chain without consuming itself such as butylated hydroxytoluene (BHT) and tocopherols (vitamin E), chelating agents that form complexes with metal ions and act as catalysts in oxidative process i.e., ethylenediaminetetraacetic acid (EDTA), and Synergistic agents those enhance the efficiency of some antioxidants i.e.,citric acid and tartaric acid (Elena M. Balboa et al., 2015). The main reason of great demand of natural cosmeceuticals is they possess numerous benefits against synthetic cosmeceuticals and have no health and safety issues. Marine environment is a rich source of highly valuable compounds those possess several cosmeceutical ingredients such as polysaccharides, polyphenols, fatty acids, several minerals, vitamins, antioxidants, bioactive peptides and enzymes (Kim, 2012).

1.3 Macro-algal biodiversity in South Australia

Sea weeds provide an attractive opportunity as a utilization resource due to their fast growth rates comparatively terrestrial plants and wider applications in natural health and cosmeceuticals. Due to potential ingredients of seaweeds their products commercialization demand have in great appeal. South Australia is one of the richest region in marine macroalgal diversity throughout the world which contain around 123 chlorophyta species (green algae), 203 Phaeophyta species (brown algae) and nearly 800 Rhodophyta species (red algae) (Womersley, 1990). The big kelp (*Macrocystis angustifolia*) and the bull kelp (*Durvillaea potatorum*) of macroalgal flora are found in the Robe (in the Southeast of South Australia).

Durvillaea potatorum, Ecklonia radiata and several red algae such as *Gracilaria* are targeted by the fishery. South Australia also called as big hotspot of macroalgal biodiversity and rich source of potential macroalgal ingredients (Phillips, 2001). Around 1200 recorded sea weed species found in South Australia in which 62% species appear to be endemic in this region. Comparatively other macroalgae, brown seaweeds (Phaeophyceae) have found to be relatively great diversity and endemism betwwen 231 species that reported in Southern Australia, in which 57 % are considered to be endemic (Womersley 1990). Consequently, South Australia is known for high degree described species richness and for endemism (Phillips, 2001). Due to favorable climate and clean nutrient rich water marine flora grows abundantly in South Australia. Several industries developing by utilization of harvested beach cast seaweeds for the production of hydrocolloids, agricultural products, and health-care products by using bioactive ingredients from sea weeds (Lee, 2010).

The table 1.1 showing the representation of macro algal orders which found in South Australia. According to the recent survey of October 2011, *Ecklonia radiata*, *Scytothalia dorycarpa*, *Greville, Cystophora spp*., *C. moniliformis*, *C. platylobium*, *Durvillaea potatorum*, *Macrocystis angustifolia* were the most common species found in South Australian waters in which around 7% species of the wrack (Womersley, 1990). *Callophyllis lamber* and *Hymenena affinis* and green algae generally belongs to *Codium* or *Caulerpa genera* and found in negligible proportion (<1%) of the wrack perhaps due to their little morphology and shortage in the near-shore waters (Womersley, 1990).

However, the data related to yearly production of definite species in ecosystems alongside the coastline, the availability of all species inside the beach cast wrack is scarce at the current or probable harvest sites most importantly as a commercial point of view. Meanwhile, is the Rivoli Bay, Beachport, SA, as an appropriate wrack where significant amount of seaweeds are deposited throughout the year. They are easily available and commercially harvested under license for the

manufacture of low value products for instance fertilizers and animal feeds. According to recent research on derived functional food ingredients from seaweeds, brown seaweeds are found to be richest source of nutraceuticals with a range of bioactive compounds, primarily sulfated polysaccharides (fucoidan), phenolic compounds (phlorotannin), and carotenoids (fucoxanthin). Moreover, they also exhibit polyunsaturated fatty acids (PUFAs), such as omega-3 fatty acids and bioactive peptides (Holdt and Kraan 2011). These bioactive compounds hold various biological properties including antioxidant, anti-HIV, anticancer, antidiabetic, antimicrobial, anticoagulant, antivirus, antitumor, antiinflammatory, immunomodulatory, dietary fiber, gastric protective effects, and blood lipid or cholesterol reduction (Mohamed et al. 2012; Jiménez-Escrig et al. 2013).

Table 1.1: Representation of macro algal orders in South Australia (Womersley, 1990)

Class	Order	Genera	Species
Chlorophyta	Bryopsidales	Caulerpa	19
		Codium	16
Phaeophyta	Fucales	15	61
	Chordariales	27	44
	Dictyotales	15	41
	Sphacelariales	6	26
	Ectocarpales	13	21
	Sporochnales	8	12
Rhodophyta	Ceramiaceae	53	199
	Rhodomelaceae	59	167
	Gigartinales	46	122
	Cryptonemiales	49	105
	Nemaliales	21	62
	Delesseriaceae	24	50
	Rhodymeniales	17	40
	Dasyaceae	3	32
	Gelidiales	5	9

1.4 Studies of sea weeds used in health care & cosmeceuticals

Nowadays, as per the great demand and market of nature based cosmeceuticals, marine algae are widely used in personal health and skin care cosmetic products due to their novel bioactive compounds. The extract of algae has been used by two different ways as an excipient (stabilizer) or as a therapeutic agent itself (Morton, 2013). In general, macro algae has been divided into three groups, green algae (Chlorophyta, red algae (Rodhophyta) and brown algae (Phaeophyta) and some of these algal compounds possess cosmetic and pharmaceutical properties which lead to origin cosmeceuticals (Morton, 2013). As per previous studies algae are the major sources of marine antioxidants. Comparatively, red and green algae the brown algal extract possess more effective antioxidant activity because of phlorotannin content, phloroglucinol derivatives, polysaccharides and carotenoids fraction (Elena M. Balboa et al., 2015). Algal solvent extract have been explored as novel photo-protective compound for pharmaceutical and cosmeceutical product formulation. Several algal bioactive ingredients have shown activity sargaquinoic acid and sargachromenol from *Sargassum sagamianum*, dieckol from *Ecklonia cava*, or fucoxanthin from *Sargassum siliquastrum* (Guinea et al., 2012).

Various species of red algae such as *Porphyra atropurpurea* and *Chondrus crispus* shows regeneration properties so they have been used to treat wounds and burns (Charlier and Chaineux, 2009). According to screening of 43 seaweed species to determine antibrowning effects, some seaweeds called *Endarachne binghamiae*, *Schizymenia dubyi*, *Ecklonia cava*, and *Sargassum siliquastrum* inhibited cellular melanin production, tyrosinase activity and did not show toxicity (Elena M. Balboa et al., 2015). Various polysaccharide components, particularly those extracted from brown algae (*Ecklonia cava*, *Sargassum stenophyllum*, *S. hornery*, *Costaria costata*) decrease melanoma cell tumor growth (Ale et al., 2011a).

Several marine algae anticipated to a great source of bioactive ingredients with several health benefits and in treatment of allergic diseases (Thomas and Kim, 2013). As per previous studies by (Wang et al., 2009) the novel antioxidants from Icelandic marine sources are used for food additives, for functional ingredients and for nutritional supplements. From different seaweed species in Hvassahraun total polyphenolic content and antioxidant activity has been determined by using three different antioxidant assays. These obtained antioxidants assay have been showed an innovative alternative to improve oxidative stability, flavor quality, nutritional value of food products, utilization in functional foods and in nutritional supplements.

Seven brown seaweeds *(Fucus vesiculosus, Laminaria digitata, Alaria esculenta, Fucus serratus, Saccharina latissima, Laminaria hyperborea,* and *Ascophyllum nodosum*), two red seaweeds *(Palmaria palmata* and *Chondrus crispus),* and one green seaweed *(Ulva lactuca)* contain polyphenolic compounds with bioactive property such as antioxidant activity. In contrast, brown seaweeds generally contained higher amounts of polyphenolic compounds rather than red and green seaweeds, particularly in *F. Vesiculosus, F. Serratus,* and *A. nodosum*.

A high correlation was observed between total polyphenolic content and antioxidant activity of the seaweed species (Wang et al., 2009). Table 1.2 illustrates the South Australian sea weed species with high valuable active compounds those possess commercial applications in cosmeceuticals and health care products and Table 1.3 describes regarding cosmetic product and their manufactures those sourced from marine ingredients having antioxidant activities.

Table 1.2 Bioactive compounds derived from well documented South Australian marine sources having potential commercial applications (Stengel et al., 2011, Holdt, 2011)

Bioactive ingredients	**Applications**	**Well documented Southern Australian sources**	**Southern Australian seaweed species**
Fucoidan	Anti-coagulant Anti-cancer Anti-aging Contraceptive Microbicidal Anti-inflammatory	*Scytosiphon lomentaria* *Macrocystis pyrifera* *Undaria pinnatifida* *Durvilleae Antarctica*	Most brown algae, *Chordaria cladosiphon* 2 *Cladosiphon* spp. *Macrocystis angustifolia* *Durvilleae potatorum* Many *Sargassum* spp. *Ecklonia radiata*
Alginate	Thickener and emulsifier Fire and water proofing Paper and textile sizing Drug delivery systems	*Durvilleae potatorum* *Durvilleae Antarctica*	Various kelp, *Ecklonia radiata* *Macrocystis angustifolia*
Carrageenan	Thickener and emulsifier Lubricant Anti-viral		Various red algae, >6 *Gigartina* spp.
Agar/agarose	Gelling agent Thickener and emulsifier	*Gracilaria chilensis* *Pterocladia lucida*	Certain red algae, ~6 *Gelidium* spp. ~7 other *Gracilaria* spp.
Haloperoxidases	Halogenating organic compounds	*Ulva compressa*	Diverse algae, ~10 other *Ulva* spp. Several *Corallina* spp.
Terpenoids	Anti-cancer Anti-fouling agents Pest-controlling agents Anti-viral		Diverse algae, ~14 genera belonging to Dictyotaceae >7 *Laurencia* spp.
Polyphenols	Anti-fouling agent Anti-cancer Anti-bacterial Skin-whitening		Various algae, especially brown algae, Many *Cystophora* spp. *E. radiate*
Fucoxanthin	Anti-obesity Anti-aging		Many brown algae
Halogenated compounds	Anti-fouling agent Anti-cancer Anti-bacterial	*Sargassum fallax*	Mostly red, some brown, few green algae, >7 *Laurencia* spp.

Table 1.3 Polyphenolic cosmetic products which derived from marine sources.

Source	Ingredient	Action	Product name	Manufacturers	References
Ecklonia cava	Polyphenols & phlorotannins	Antioxidant	PC-Ecklonia cava	Biopure's	http://www.amazon.com/BioPure-Ecklonia-Cava-Polyphenol-Phlorotannin/dp/B010TUFDDE
Ecklonia cava	Polyphenols & phlorotannins	Antioxidant	Fibronol®	Allergy Research group®	http://www.allergyresearchgroup.com/fibronol-150-vegetarian-caps
Ecklonia cava	Polyphenols & phlorotannins	Antioxidant	Fiboboost ®	Allergy Research group®	http://www.allergyresearchgroup.com/fibronol-150-vegetarian-caps
Ecklonia cava	Polyphenols & phlorotannins	Antioxidant	Seanol	Seanol®	http://www.seanolinside.com/products.html
Ecklonia cava	Polyphenols & phlorotannins	Antioxidant	Fiboboost	Nutria Cology®	http://au.iherb.com/Nutricology-FibroBoost-75-Veggie-Caps/7777
Ascophyllum nodosum & Fucus vesiculosus	Polyphenols	Antioxidant	InSea^2	Bestivite^{TM}	http://www.amazon.com/InSea2-250mg-120-Vegetarian-Capsules/dp/B007G42484

1.5 High valuable products found in macro algae

Sea weeds contain various bioactive compounds that have potential applications in health care and cosmeceuticals. Because of these active ingredients sea weed industry launched a variety of products which have estimated annual income of US $ 5.5-6 billion. According to the recent survey of food and agriculture organization of United Nation food products contribute by US $5 billion and rest of income sourced from sea weeds extracted hydrocolloids, fertilizers and animal feed

additives. Australia is known as main importer of seaweed products, which imported 5000 T valued at over AUS$17 million during the 2008–2009 financial year (Lee, 2010).Well developed countries like Australia are the competitive manufactures of high value products due to utilization of its advance downstream processing capabilities (Winberg, 2009). Approximately 30% of the seaweeds bioactive products isolated in 2009 in which 3,280 structures are reported at this time (Holdt, 2011).

1.5.1 Polysaccharides

Fucoidans: Fucoidan contain sulfated polysaccharides which present in various macroalgae and macroalgal blends and widely used in cosmeceutical formulations for many decades because of its viscosity controlling, skin conditioning and bioactive applications (Morton, 2013). Fucoidans have high percentage of L-fucose which is acetylated or sulfated and is mainly found in many brown algal species such as *Laminaria japonica, Fucus vesculosus, Undaria pinnatifida, Cladospiphon okamuranus,* and *Hizikia fusiforme.*

The fucoidan extract of F. vesiculosus after purification used in creams, lotions, anti-ageing cosmetics and provide anti-wrinkle benefits through inhibition of matrix enzymes aligned with hyaluronidase, heparanase, phospholipase A2, tyrosine kinase and collagenase (Morton, 2013). The fucoidan which extract from brown algae acquire antiviral, anticancer, immunomodulatory, anti-inflammatory, anti-lipidemic and other activities (Ale et al., 2011b) and (Jiao et al., 2011) .

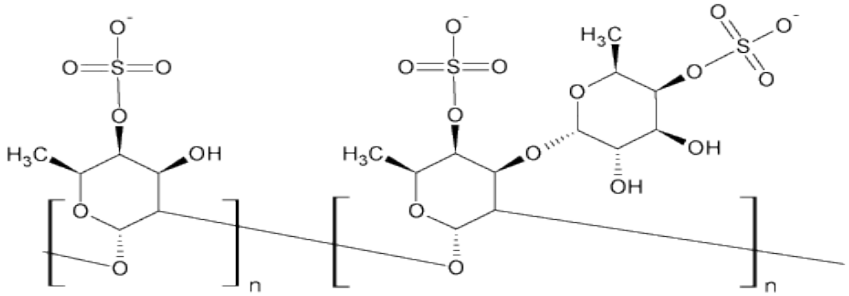


Figure 1.1 Patankar model for the common structure of fucoidans (www.esciencecentral.org)

The hydrocolloids those derived from sea weeds known as phycocolloids and have global market more than US $1 billion in 2009 whereas half of the income contributes by non whole food seaweeds products (Bixler and Porse, 2010). Phycocollides are high molecular weight structural polysaccharides found in cell walls of macroalgae and make solutions after dissolving in water. Sulfated glacatans agar, carrageenan and alginate from brown algae are the commercial high valuable types of phycocolloids (Stengel et al., 2011).

Agar: Agar is another important component of algae which is used in the food and cosmetic industries. *Gelidium* and *Gracilaria* are primary sources of agar and may account up to 35% of dry weight of algae (Kapraun et al.). Agar molecules contain less amount of sulfate esterification (<6% w/w) (Karina H. M. Cardozo, 2007). In general point of view, the strong gel is formed if the substitution of sulfate is low and those compounds which do not contain sulfate are termed as agarose. Molecular weight, pyruvate and methoxy substitution are the other physicochemical factors that affect the gelling properties (Barros et al., 2013). Low quality agars used as thickeners, emulsifiers, in gelling agents and in foods comparatively, high quality agars used in the preparation of culture media to propagate plant and bacteria and gels are used for isolation and examination of micro molecules (DNA, RNA, Proteins) in molecular biology experiments or in forensic labs (Selby, 1993).

Carageenans: Carageenans is a generic name for a family of hydrophilic polysaccharides with highly effective thickening and gelling properties which used in the food industry, in emulsifiers, stabilizers, in cosmetics, shampoos, skin creams, toothpastes and various other commodities such as air fresheners and personal lubricants (McHugh, 2003). Red algal extract and carageenans are known for their in vivo antiviral activity against sexually transmitted diseases (Bourne et al., 1999). *Kappaphycus alvarezii*, *Eucheuma spinosum, J.Agardh* and *Gigartina spp*. are the most commonly

used species for commercial production due to the nature and content of carrageenans including other advantages like their capacity for aquaculture (McHugh, 2003).

Alginate: Alginate or alginic acid is a class of phycocolloid which obtained from the brown seaweeds. It is a structural polymer consisting of backbone of D-mannuronic acid and L-guluronic acid residues and may account up to10-30% of the plant's dry weight (Cardozo, 2007). It is extracted from various common species of algae which are *Saccharina* (formally *Laminaria*), *Macrocystis, Ascophyllum, Ecklonia*, and *Durvillaea* (Lee, 2010). Alginates have ability to form viscous solutions and gels because it readily absorbs water (Cardozo, 2007). Due to its several beneficial characteristics it commonly used in various industrial applications such as sizing of paper and textiles, water and fire-proofing of fabrics, an additive to dehydrated products, a thickening agent in ice creams, in jellies, soups, beverages, and cosmetics as well as in drugs in the pharmaceutical industry (Lee, 2010).

1.5.2 Proteins

As compared to other valuable products of sea weeds proteins have little commercial significance, although some researchers gave some reviews on the composition and potential applications of sea weeds. The brown algae contain only 3-15% dry weight of protein whereas red and green sea weeds account up to 10-47% of dry weight of protein (Fleurence, 1999). Sea weeds proteins are used in research industry for utilization of light-harvesting cellular machinery called phycobiliproteins that take part in the photosynthetic pathways of red seaweeds and cyanobacteria. In many red algae the most common phycobiliprotein is known as R-phycoerythrin, which present in the cells of some species when grown under optimal conditions (Glazer, 1994). These florescent phycobiliproteins

used in various applications such as flow cytometry, cell sorting, histo-chemistry and also used as food dye (Glazer, 1994, Fleurence, 1999). Meanwhile, seaweeds are the big source of various useful enzymes and these enzymes (haloperoxidases) react readily with organic compounds to form halogenated products that help to endorse the bioactivity of organic compounds that produce useful pharmaceutical and industrial products (Butler, 2009).

Haloperoxidases have been derived from all three groups (red, brown and green algae) of macroalgae to produce volatile halogenated compounds those used in antifouling system and possess antimicrobial properties (Wever, 2001). There are twenty one species of arctic brown, red and green seaweeds detected for haloperoxidase activity, in which highest iodoperoxidase activity detected in the green algae *Acrosiphonia sonderi*, *Ulva compressa* and the highest bromoperoxidase activity detected in the brown algae *Saccharina latissima* (Mehrtens, 1994).

1.5.3 Terpenoids

Terpenoids are the biggest and most common class of secondary metabolites which abundantly found in higher plants such seaweed species, in insects and in microorganisms (Morton, 2013). Many macroalgal cytotoxic metabolites have been isolated from terpenoids such as dehyrothysiferol from the red seaweed *Laurencia viridis* from the Canary Islands, which is a triterpenoid polyether which was used to stimulate apoptosis in human breast cell lines (Pec, 2003). Sesquiterpene caulerpenyne is the main secondary metabolite of *Caulerpa taxifolia* a genera which particularly contained anti-proliferative activity against a broad range of human cancer cell lines (Fischel, 1995). Along with various biological activities of terpenoids of macroalgae, they show toxicity against various marine and terrestrial organisms such as mosquito larvae, cockroaches, epiphytes, and parasitic worms. For this reason, terpenoids are potentially used in household and aquaculture pest

controlling agents (Smit, 2004). Carotenoid, fucoxanthin is tetra-terpenoid pigment that found in brown algae and used for dietary administration for the prevention and treatment of obesity and metabolic syndromes. Moreover, it reduces the risk of Type II diabetes and helps in decreasing body and liver fat (Miyashita, 2008). Fucoxanthin and other algal carotenoids have capability to stimulate apoptosis in number of human cancer cell lines, ability to prevent the wrinkle formation and power to reduce the epidermal hypertrophy (skin thickening, which causes wrinkle formation). Therefore, these bioactive compounds have potential applications in cosmeceuticals such as antiageing creams (Urikura, 2011).

1.5.4 Halogenated compounds

Marine algae are the house of halogenated metabolites with potential commercial value (Cabrita et al., 2010). Many macroalgal secondary metabolites possess cytotoxic activites due to their halogenated nature especially when they incorporated with bromine and chlorine elements. Red algae contain 90% of secondary metabolites in which green algae possess 7% and only 1% in brown algae (Cabrita, 2010). Halogenated macroalgae contain antimicrobial properties (e.g.*Laurencia majuscula*) and used for natural antifouling agents for boats and marine structures coating (Vairappan, 2003). One Australian group of Sydney searched on halogenated furanones or fimbrolides those obtained from the red seaweed *Delisea pulchra* and act as antagonists of molecules that helps in sensing recognition systems in bacteria and preventing the colonization of bacteria by non-toxic mechanism of action (De Nys, 1995).

1.5.5 Polyphenols/Phlorotannins

Among various classes of algal polyphenols, phlorotannins are important bioactive compounds that composed of several phloroglucinol (1,3,5-trihydroxybenzene) units which linked together by different ways (Thomas and Kim, 2011). According to the nature of structural linkages and phloroglucinol units with different hydroxyl groups, phlorotannins mainly subdivided into six specific groups: phlorethols, fuhalols, fucols, fucophlorethols, eckols and carmalols (Thomas and Kim, 2011). The distribution of phlorotannins in brown seaweeds varied from species to species and affected by size, age, tissue type, salinity, season, nutrient levels, light intensity and water temperature of different seaweeds (Sathya et al.). Along with other polyphenolic compounds, phlorotannins show several remarkable properties on biological systems, namely antioxidant (Sathya et al.), anti-inflammatory (Liu et al., 2014), anti-allergic (Barbosa et al., 2014b), antimicrobial (Eom et al., 2012), anticancer (Kim et al., 2011) and antidiabetic (Lee and Jeon, 2013) activities.

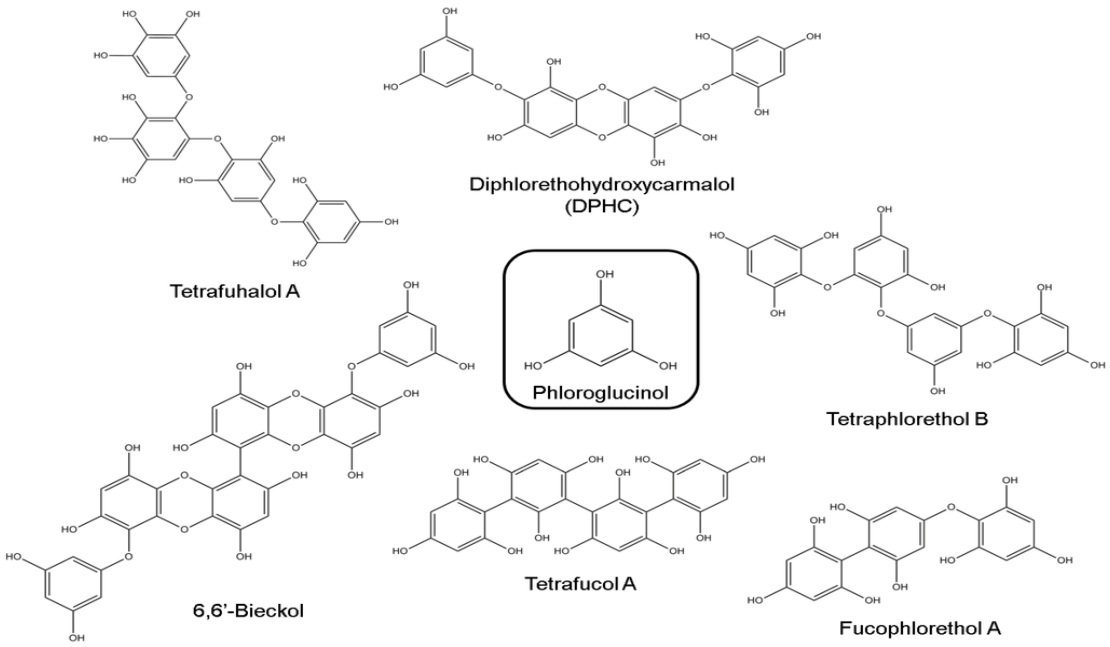


Fig. 1.2 The chemical structures of phlorotannin compounds isolated from brown seaweeds (Wijesinghe et al., 2011)

Phlorotannins promise a great deal of bioactive compounds especially from brown algae. Phlorotannins are found in kelps of *Ecklonia, Cystosphora* which found in coastline of South Australia. Phlorotannins have ability to stimulate de-pigmentation or whitening of skin by the inhibition of melanogenesis (melanin is produced by cells known as melanocytes) which regulated by enzyme tyrosinase (Wijesinghe et al., 2011). Several phlorotannins have been found to inhibit the activity of enzyme tyrosinase such as 7-phloroeckol and dieckol from *Ecklonia cava* (Heo, 2009) phloroglucinol derivatives from *Ecklonia stolonifera* (Kang, 2004).

The phlorotannins of *E. radiata* have been used for antifouling agents that inhibit the settlement and growth of the gametes of the fouling green seaweed *Ulva lactuca*. Phlorotannins are common for antibacterial activities particularly against food borne pathogens including methicillin-resistant *Staphylococcus aureus*. Various types of phlorotannin compounds derived from different brown sea weeds *E. cava, E. radiata, E. bicyclis and E. kurome* as shown in figure 1.2 (Wijesinghe et al., 2011). In *E.cava* phlorotannins dieckol and phlorofucofuroeckol was isolated and related with the growth of chief central neurotransmitters in the brain of a selected animal model, mainly of ACh, with the inhibition of AChE (Myung et al., 2005).

From *Ecklonia stolonifera* various other types of phlorotannins were isolated called Eckol, dieckol, 2-phloroeckol and 7-phloroeckol which inhibit AChE and eckstolonol and phlorofucofuroeckol A inhibited both AChE and BuChE (Yoon et al., 2008). From *Ishige okamurae* Yoon and his colleagues purified phloroglucinol, 6,6′-bieckol and diphlorethohydroxycarmalol (DPHC), determined their ChE inhibitory capacity and demonstrated that 6,6′-bieckol and DPHC showed effective AChE, moderate BuChE inhibitory effects and 6,6′-bieckol acts as a non-competitive inhibitor according to Lineweaver-Burk plot studies of enzyme kinetics (Pangestuti and Kim, 2011). Dieckol purified from *E. cava* displayed anti-inflammatory properties and in result it was able to

lessen the expression and release of pro-inflammatory mediators and cytokines, such as PGE2, IL-1β and TNF-α via down-regulation of nuclear factor κB (NF-κB), p38 kinase activation or inhibition of ROS signal within microglial cells (Jung et al., 2009). In recent studies, it was evaluated that neuro-protective potential of DPHC based on its antioxidant capacity and showing that DPHC was able to protect cells from oxidative stress-induced neurotoxicity, that can offer health benefits, including prevention of neurodegenerative diseases (Heo et al., 2012).

Methanolic extract of *Eisenia bicyclis* is used for study of neuroprotective effects with alongwith purified phlorotannins on Aβ-induced toxicity in PC12 cells mediated by the suppression of intracellular ROS and the reduce the level of Ca2+ ions. As a result, 7-phloroeckol and phlorofucofuroeckol-A were strong neuroprotective agents. On the other hand, eckol exhibited a weaker effect. These results suggest that the size of the molecule and quantity of hydroxyl groups in phlorotannins' compounds are important features which reveals their neuroprotective effects against Aβ-induced cytotoxicity (Ahn et al., 2012).

The antioxidant activity obtained from phlorotannins may be the result of specific scavenging of radicals formed during peroxidation, scavenging of oxygen/nitrogen-containing compounds or metal-chelating ability (Li and Kim, 2011). Several phlorotannin components, including fucophlorethol A, tetrafucol A and trifucodiphlorethol A, purified from *Fucus vesiculosus* was able to scavenge reactive carbonyls, by inhibiting the production of AGEs (Figure 1.3) (Liu and Gu, 2012).

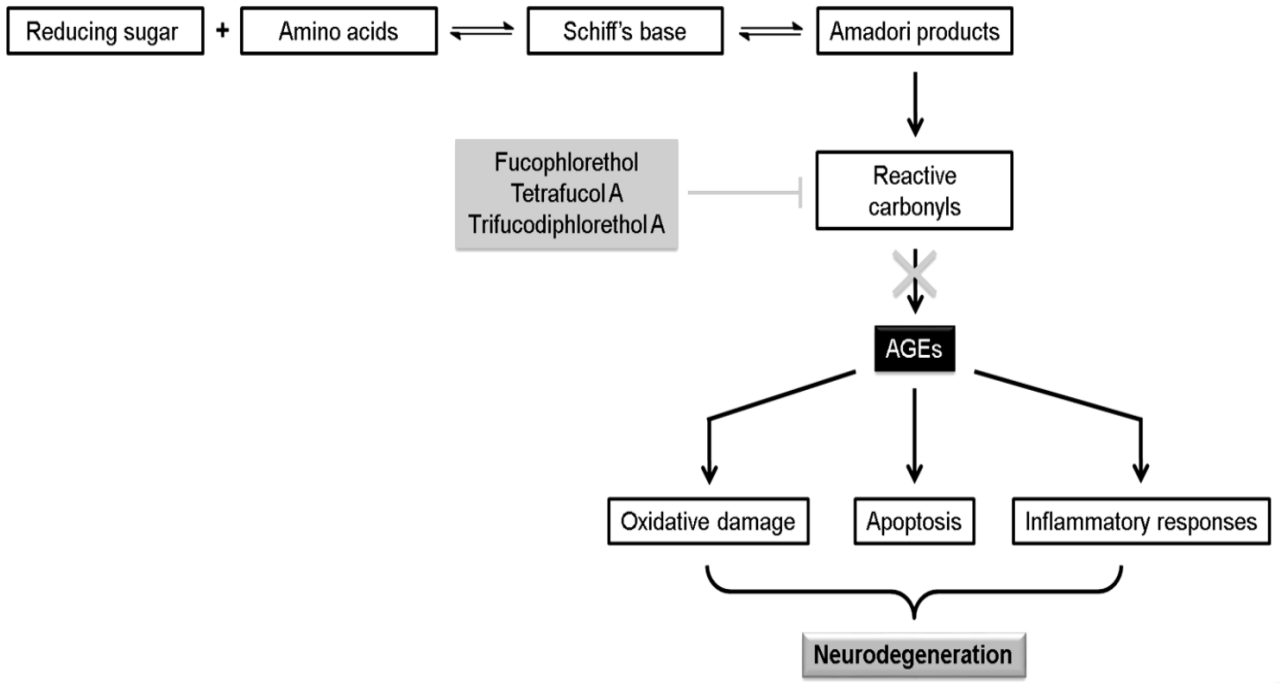


Figure 1.3 Inhibition of the production of glycation end products (AGEs) by few polyphenolic compounds by scavenging activity of reactive carbonyl intermediates (Barbosa et al., 2014a)

Although different methods applied for the prevention of progression of neurodegeneration, ChE inhibitors are still the most effectual approach to the suggestive treatment of neurodegenerative disorders. According to the recent evidence it was shown that AChE inhibited by phloroglucinol and dibenzo [1,4] dioxine-2,4,7,9-tetraol and eckol, phlorotannins purified from *Ecklonia maxima* (Kannan et al., 2013). Both dibenzo [1,4] dioxine-2,4,7,9-tetraol and eckol proved to be stronger AChE inhibitors than phloroglucinol because of their large molecular size and the presence of a larger number of hydroxyl groups, which are able to modulate the interaction with AChE and consequent inhibition of the enzyme (Kannan et al., 2013). The previous data suggest that seaweeds may offer important sources of polyphenolic compounds with potential application as pharmaceutical or nutraceutical agents for prevention and control of neurodegenerative processes by different pathways and also posses antioxidant activities (Figures 1.4 and 1.5).

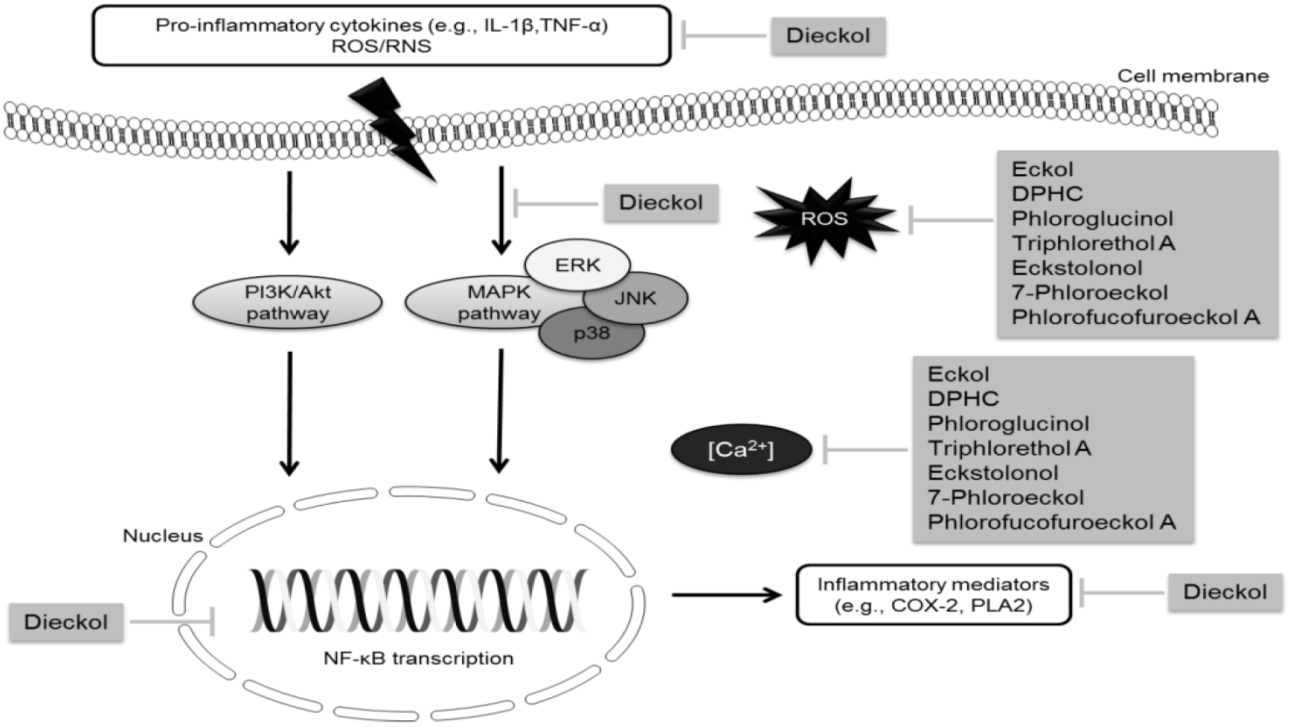


Figure 1.4 Phlorotannins involved in neuroprotection mediated by anti-neuroinflammatory and antioxidant mechanisms (Barbosa et al., 2014a)

The potential antioxidant activities of three phlorotannins (phloroglucinol, eckol and dieckol) purified from *Ecklonia cava* were investigated to find their potential value as the natural products for foods or cosmetic application. As a result, these three phlorotannins showed potential inhibitory effect on H_2O_2-mediated DNA damage and on harmful free radicals which can be used as antioxidants in cosmetics, foods and in drug industry (Ahn et al., 2007). The phlorotannins of two edible seaweeds *Laminaria* and *Porphyra* sp. have been reported to decrease the threat of intestinal or mammary cancer, anti-carcinogenicity on cell proliferation (Yuan and Walsh, 2006). Xiaojun and his colleagues examined 27 seaweeds species for antioxidant activities from which only 15 seaweed species had good ability to scavenge hydroxyl radicals and *Gelidium amansii, Gloiosiphonia capillaris, Polysiphonia urceolata, Sargassum kjellmanianum, Desmarestia viridis,* and *Rhodomela teres* showed the strongest antioxidant activities (Yan et al., 1998).

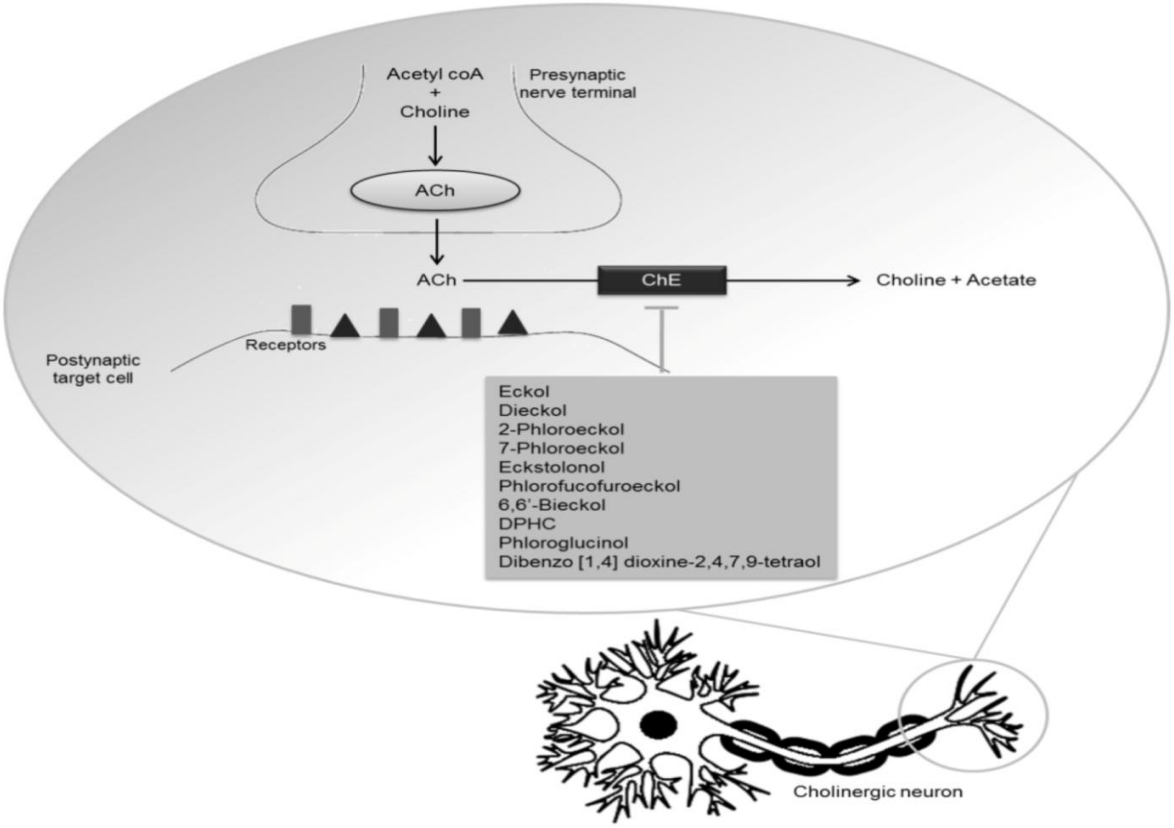


Figure1.5. Phlorotannins with ChE inhibitory activity (Barbosa et al., 2014a)

The 70% ethanol crude extract (phlorotannins) of *Sargassum palladium* after examination proved as good source of natural antioxidant because it showed highest antioxidant activity (0.52 μmol $FeSO_4$equivalent/mg extract) (Ye et al., 2009). Phlorotannins extracted from *Ecklonia cava* contained significant activities such as antioxidant, anticancer, antidiabetic, anti-human immunodeficiency virus, antihypertensive, matrix metalloproteinase enzyme inhibition, hyaluronidase enzyme inhibition, radioprotective, and antiallergic activities (Wijesekara et al., 2010).

1.6 Extraction methods of phlorotannins

With the presence of various extraction protocols of phlorotannins from the algae, the isolation procedures of phlorotannins are widely variable (Hagerman, 1988). It has been noted that tannins extraction by acetone enhance the total yield by inhibiting interactions between tannins and proteins (Hagerman, 1988) and due to breaking of hydrogen bonds between protein tannin complexes (LJ., 1989). Organic solvents called acetone and ethanol mostly used to extract phlorotannins from *F. vesiculosus* (KW, 1986). The key feature of phlorotannins that they oxidized rapidly and in various studies it shown that extracted phlorotannins are acetylated with aceticanhydride-pyridine to protect them from oxidation (Stern et al., 1996).

To extract phlorotannins from *Sargassum pallidum* 70% ethanol have been used (Hong Ye et al., 2009), 50% methanol was used to isolate polyphenols from three algae such as *Padina antillarum, Caulerpa racemosa* and *Kappaphycus alvarezzi* (Devi et al., 2011). In case of *Fucus vesiculosus* mixture of solvents called methanol/water (80:20, v/v), ethyl acetate/water (80:20, v/v), acetone/water (70:30, v/v), ethanol/water (80:20, v/v), and ethanol/water (50:50, v/v) have been used to extract polyphenols (Wang et al., 2012).

From *Lamineria japonica* ultrasonic extraction method have been used to extract polyphenols by using water, methanol and various other solvents such as1-Ethyl-3-methylimidazolium tetrafluoroborate ([Emim][BF4], 98%) 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF4], 98%), 1-hexyl-3-methylimidazolium tetrafluoroborate ([Hmim][BF4],98%), 1-butyl-3-methylimidazolium chloride ([Bmim][Cl],98%) (Zhao, 2006). Methanol used for isolation of phlorotannins from *Sargassum marginatum, Padina tetrastomatica* and *Turbinaria conoides* (S. Kumar Chandini et al., 2008). For *Ecklonia stolonifera* methanol, ethyl acetate, butanol and dichloromethane were used to get polyphenols (Kim et al., 2009a) and methanol was used to isolate

phlorotannins from *Cystoseira trinodis* (Sathya et al.). To summarize this data above table 1.4 provides the information regarding different extraction methods of some seaweed species.

Quantization of Total Phenolics

Phlorotannin assay has been used to determine the total phenolic content (TPC) by using Folin-Ciocalteu reagent with minor modifications for *Ulva* species. Folin-Ciocalteu reagent is followed by the addition of Na_2CO_3. This assay was carried out in microplate. In this assay, gallic acid was used as the standard reference.TPC was expressed as mg gallic acid equivalents per gram of dried extract of seaweed (mg GAE g^{-1}) (Farasat et al., 2014).

Table 1.4 Summary of different extraction methods of phlorotannins from various seaweeds

Source	Extraction method	Solvents used	References
F. vesiculosus	Solvent extraction	Acetone and ethanol	(KW, 1986)
Sargassum pallidum	Solvent extraction	70% ethanol	(Hong Ye et al., 2009)
Padina antillarum, Caulerpa racemosa and *Kappaphycus alvarezzi*	Solvent extraction	50% methanol	(Devi et al., 2011)
Fucus vesiculosus	Solvent extraction	Methanol/water(80:20,v/v), ethyl acetate/water (80:20, v/v),acetone/water (70:30, v/v),ethanol/water (80:20, v/v), and ethanol/water (50:50, v/v)	(Wang et al., 2012)
Lamineria japonica	Ultrasonic extraction	Ethyl-3methylimidazolium tetrafluoroborate([Emim][BF4], 98%) 1butyl3methylimidazolium tetrafluoroborate ([Bmim][BF4], 98%),1hexyl3methylimidazolium tetrafluoroborate([Hmim][BF4],98 %), 1-butyl-3-methylimidazolium	(Zhao, 2006)

		chloride ([Bmim][Cl],98%), methanol, water	
Sargassum marginatum, Padina tetrastomatica and *Turbinaria conoides*	Solvent extraction	Methanol	(S. Kumar Chandini et al., 2008)
Ecklonia stolonifera	Solvent extraction	Methanol, ethyl acetate, dichloromethane and butanol	(Kim et al., 2009a)
Cystoseira trinodis	Solvent extraction	Methanol	(Sathya et al.)

1.7 Antioxidant Activity

Antioxidants are compounds which are man-made or natural and inhibit the oxidation of lipids or other molecules through inhibiting the propagation of oxygen chain reactions by prevention and repair of damaged cells by oxygen (Javanmardi and Kubota, 2006). Ultraviolet radiations, ionizing radiations, various chemical and metabolic processes are responsible for the production of ROS in the cells (Askarova et al., 2011). In the form of byproducts such as superoxide ions, hydrogen peroxide, hydroxyl radicals ROS produced during human's natural metabolism. The defense system of humans have combated and reduced oxidative damage but unable to eliminate the harmful reactive substances completely (Teixeira et al., 2009).

Therefore, an adequate intake of natural antioxidants in food or via cosmeceuticals or healthcare products has been used to provide protection of macromolecules against oxidative damage in cells. Hence, antioxidant compounds such as polyphenols/phlorotanins from marine sources received great interest in field of research due to their valuable biological activities. Various in vitro methods

have been developed to analyze the potential of natural antioxidants from bioactive compounds of seaweeds or from their extracts. In vitro methods can be divided into two main groups which are 1) hydrogen atom transfer reactions such as Oxygen Radical Absorbance Capacity (ORAC), Total radical trapping antioxidant potential (TRAP) and β-carotene bleaching; 2) Electron transfer reactions like trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP), α-diphenyl-β-picryl-hydrazyl radical scavenging assay (DPPH), superoxide anion radical scavenging assay, hydroxyl radical scavenging assay, nitric oxide radical scavenging assay and total phenol assay have been reported in the literature (Narasimhan et al., 2013). All these methods are very popular and used to determine the antioxidant, antimicrobial and anti-proliferative activities of various sea weeds such as *Gracilaria corticata*, *Enteromorpha antenna*, *Enteromorpha linza* (Salazar et al., 2008).

Table 1.5 shows the example of common bioactive ingredients that extract from marine resources having antioxidant activities. It has been reported that three phlorotannins phloroglucinol, eckol and dieckol purified from brown algae *Ecklonia cava* found to possess antioxidant activities (Wijesinghe, 2011). Moreover, potential antioxidant activities were reported from phlorotannin derivatives of E.cava and instead of this 6,6-bieckol, dieckol and fucodiphloroethol (Wijesinghe, 2011). Extracts from 48 marine macroalgae species (17 Chlorophyta, 8 Phaeophyta and 23 Rhodophyta) from the coasts of Yucatan and Quintana Roo (Mexico) were examined for antioxidant activities. All species exhibited a DPPH radical scavenging activity but three species such as *Avrainvillea longicaulis*, *Chondria baileyana* and *Lobophora variegate* possess great antioxidant potential with very low oxidation index EC_{50} (1.44 ± 0.01, 2.84 ± 0.07 and 0.32 ± 0.01 mg mL^{-1}, respectively), significantly equivalent to EC_{50} of some commercial antioxidants such as α-tocopherol and ascorbic acid. As a result, some macroalgae could be considered for future applications in medicine, food production or cosmetic industry (Zubia et al., 2007). Solvent extracts

of *Kappaphycus alvarezii*, were investigated to find antioxidant activities in result the ethanol extract proved to be superior than synthetic antioxidants butylated hydroxytoluene (BHT). Hence, these extracts could be considered as natural antioxidants and may be useful for curing diseases arising from oxidative deterioration (Kumar et al., 2008).

Table 1.5 Bioactive ingrdients derived from seaweed species showing potential antioxidant activities

Ingredient	**Source**	**Antioxidant activity**	**Reference**
Carotenoids and other terpenoids	Microalgae, Macroalgae	Radical scavenging: DPPH, 12-DS, NB-L, AAPH, ABTS, ABAP, superoxide anion, catalyses activity in rat plasma and erythrocytes Protection against oxidation	**(Barbosa et al., 2014b)**
Phlorotannins	Brown algae	Scavenging activity against DPPH radical, hydroxyl radical and superoxide anions, protection against oxidation, ferrous and cuprous ion chelating activity, reducing activity, protection against oxidative stress in cells, protection of membrane oxidation in cells	**(Balboa et al., 2013)**
Vitamin C and E	Macroalgae and Sea grasses	Scavenging activity against DPPH radical, reducing power, endogenous enzyme activity of superoxide dismutase and glutathione peroxidase	**(Suleria et al., 2015)**
DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABAP: 2,2'-azo-bis-2-amidinopropane; ABTS: 2'azinobis(3-ethylbenzothiazoline-6-sulfonic acid); AAPH: 2,2'-azobis(2-amidino-propane) dihydrochloride; 12-DS: 12-doxylstearic acid; NB-L: nitrobenzene with linoleic acid			

Table 1.6 Bioactive compounds from brown seaweeds having potential antioxidant activities used as cosmetics.

Brown sea weed	Active component	Activity	Reference
Ecklonia. cava	Eckol	Antioxidant	(Ahn et al., 2015)
E. cava	6,6-Bieckol, dieckol, fucodiphloroethol	Antioxidant	(Lee et al., 2010)
E. cava	Triphlorethol-A	Antioxidant	(Kang et al., 2007)
E.cava	Phlorotannins	Antioxidant	(Wijesinghe and Jeon, 2012)
S. siliquastrum	Fucoxanthin	Antioxidant	(Heo et al., 2003)
S. marginatum and P. tetrastomatica	Polyphenols	Antioxidant	(Chandini et al., 2008)
F. vesiculosus and F. serratus	Fucoxanthin	Antioxidant	(Heo et al., 2003)
E.cava and S. siliquastrum	Phlorotannins	Reduce melanin synthesis and tyrosinase activity	(Wijesinghe et al., 2011)
I. okamurae	Phlorotanins	MMP inhibitor	(Kim et al., 2009b)
E. stolonifera	Phloroglucinol	Antioxidant	(Kang et al., 2004)

To test the antioxidant activity of *Grateloupia filicina* extract compared with those of commercial antioxidants such as BHA, BHT and α-tocopherol. The methanolic extract (2 mg/mL) of *G. filicina* scavenged 82% of DPPH radicals which is almost three times higher than that of BHT. The same methanolic extract scavenged 65% of superoxide onion which is almost two times higher than that of BHT and α-tocopherol. In contrast, the extracts in chloroform and carbon tetrachloride inhibited lipid peroxidation more effectively than all commercial antioxidants tested in a linoleic acid model system (Athukorala et al., 2003).

1.8 Determination of antioxidant Activity

Free radicals have been reported to cause various diseases such as cancer, hypertension, heart diseases and diabetes by affecting the human health. Antioxidants have been shown the relevance to prevent various diseases in which free radicals are implicated (Farasat et al., 2014). Different assays have been used by different researchers to determine the antioxidant activities. In case of *Sargassum pallidum* ethanol/ ethyl acetate fraction exhibited the highest total antioxidant activity (0.52 l mmol FeSO4 equivalent/mg extract), while fractions of ethanol/ ethyl acetate and n-butanol showed the highest 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and capacity of chelating iron ions, respectively.

Microwave-assisted phlorotannins extract from *Ecklonia radiata* used to determine the antioxidant activities by Ferric reducing antioxidant power (FRAP) assay and oxygen radical absorbance capacity (ORAC) assay which showed high antioxidant activities (Charoensiddhi, 2015). To illustrate this, table 1.7 is showing the different methods of determination antioxidant activity from seaweeds. Hong Ye et al. in 2009 got high antioxidant activity from *Sargassum pallidum* by using Ferric reducing antioxidant power (FRAP) and DPPH free radical scavenging assay.

High antioxidant activity was determined from different species of *Sargassum* and two other seaweeds (*Padina tetrastomatica* and *Turbinaria conoides*) by (S. Kumar Chandini et al., 2008) and he used Phospho-molybdenum assay, DPPH free radical scavenging assay & deoxy-ribose scavenging assay to determine the antioxidant activities from the seaweeds. From *Ecklonia stolonifera* which is brown seaweed gave high antioxidant activity after tested with DDPH free radical scavenging assay (Kim et al., 2009a). As a result, FRAP, ORAC and DDPH are the best methods to determine the antioxidant activities from the seaweeds.

Table 1.7 Different methods of determination of Antioxidant activities from marine sources.

Source	Method to determine antioxidant activity	References
Ulva species (*Ulva clathrata* (Roth) C.Agardh, *Ulva linza* Linnaeus, *Ulva flexuosa* Wulfen and *Ulva intestinalis* Linnaeus)	DPPH free radical scavenging assay	(Farasat et al., 2014)
Sargassum pallidum	FRAP (Ferric reducing antioxidant power) assay & DPPH free radical scavenging assay	(Hong Ye et al., 2009)
Padina antillarum, Caulerpa racemosa and *Kappaphycus alvarezzi*	FRAP (Ferric reducing antioxidant power) assay, DPPH free radical scavenging assay & FIC (Ferrous ion chelating assay)	(Devi et al., 2011)
Fucus vesiculosus	DPPH free radical scavenging assay & FIC (Ferrous ion chelating assay), reducing assay	(Wang et al., 2012)
Sargassum marginatum, Padina tetrastomatica and *Turbinaria conoides*	Phospho-molybdenum assay, DPPH free radical scavenging assay & deoxyribose scavenging assay	(S. Kumar Chandini et al., 2008)
Ecklonia stolonifera	DPPH free radical scavenging assay	(Kim et al., 2009a)
Ecklonia radiata	FRAP (Ferrous reducing antioxidant power) assay, ORAC (oxygen radical absorbance capacity) assay	(Charoensiddhi, 2015)

1.9 Aims

1. To isolate the phlorotannins/ polyphenolic compounds from the selected seaweed species by 90% ethanol extraction method.

2. To determine the total phenolics content from the selected seaweed species and to screen the seaweed species for antioxidant activities.

1.10 Hypotheses

Seaweed species are the rich source of potential bioactive compounds and traditionally used due to their significant antioxidant properties. Therefore, selected Australian native seaweed species will have significant antioxidant compounds for the commercial applications of healthcare and cosmeceutical products.

1.11 Research Significance

The study determines that Australian sea weeds have been potentially used in health care and cosmeceuticals industries and the high valuable sea weed's polyphenols/phlorotannins exhibit antioxidant properties which have high industrial demand worldwide. New research in this area provides great opportunities for the production of health care dietary supplements, skin creams, anti-whitening, anti-cancer, anti-allergic and anti-neurological products.

Antioxidant activities have the potential to prevent the human diseases and ageing processes. Antioxidant health care products in the form of dietary intake provide significant protection of organism against free radicals. Therefore, this project promotes human health by direct consumption

of antioxidant products as supplements and drugs by using the discovered antioxidant sea weed compounds. Therefore, the research may attract great interest for cosmeceuticals, nutraceuticals and pharmaceutical companies so they fulfill the demands of the public to combat with various diseases and provide beauty as well.

Chapter 2: Materials and Methods

First and foremost, all of the reagents which used in this project were purchased from Sigma-Aldrich, USA and the chemicals were of analytical grade. High purity Milli-Q grade water was used in this study which was prepared in Mili-Q® Academic system with Quantum® EX Ultrapure Organex cartridge filter (Milipore, USA).

Chemicals:

Folin-Ciocalteu's phenol reagent, phloroglucinol, Sodium carbonate, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Sodium acetate, Acetic acid glacial, Hydrochloric acid, Ferric chloride hexahydrate, Ferrous sulphate heptahydrate, 2,2′-azobis(2methylpropionamidine)dihydrochloride(AAPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), Fluorescein sodium salt, Potassium phosphate, Potassium diphosphate and Acetone.

2.1 Australian Native seaweeds Collection

In this project, all the seventeen Australian native seaweed species *(Callophyllis lambertii, Carpoglossum confluens, Codium galeatum, Curdiea angustata, Cystophora monilifera, Cystophora moniliformis, Cystophora platylobium, Durvillaea potatorum, Ecklonia radiata, Macrocystis angustifolia, Macrocystis pyrifera, Melanthalia obustata, Phacelocarpus sp., Phyllospora comosa, Sargassum sp., Sargassum axillaris, Seirococcus axillaris*) were collected from freshly deposited beach-cast seaweed in Rivoli Bay, Beachport, South Australia on 27th August 2013 and identification of all seaweeds is confirmed by the State Herbarium of South Australia. But the three brown algae called *Durvillaea potatorum, Macrocystis pyrifera* and *Ecklonia radiata* were collected several times by Peng Su and by Dr. Andrew from Rivoli Bay,

Beachport, SA. Recently, these three seaweeds were collected in 22nd March, 2016. Table 2.1summarize the list of collected seaweeds, their place of collection along with their collection dates. The collected seaweeds were rinsed in fresh water to remove visible surface contaminants and placed on mesh racks to dry for few days under sunlight. The dried seaweeds were blended (Blendtec, USA), then passed through a 0.25-mm sieve then dried in an oven at 45 °C. The seaweeds powder was stored at −20 °C for further analysis.

Table 2.1: The list of seaweeds collected along with their collection dates and place.

Samples	**Date of collection**	**Place of Collection**
Callophyllis lambertii	27th August 2013	Rivoli Bay, Beachport, SA
Carpoglossum confluens	27th August 2013	Rivoli Bay, Beachport, SA
Codium galeatum	27th August 2013	Rivoli Bay, Beachport, SA
Curdiea angustata	27th August 2013	Rivoli Bay, Beachport, SA
Cystophora monilifera	27th August 2013	Rivoli Bay, Beachport, SA
Cystophora moniliformis	27th August 2013	Rivoli Bay, Beachport, SA
Cystophora platylobium	27th August 2013	Rivoli Bay, Beachport, SA
Durvillaea potatorum	22nd March 2016	Rivoli Bay, Beachport, SA
Ecklonia radiata	22nd March 2016	Rivoli Bay, Beachport, SA
Macrocystis angustifolia	27th August 2013	Rivoli Bay, Beachport, SA
Macrocystis pyrifera	22nd March 2016	Rivoli Bay, Beachport, SA
Melanthalia obustata	27th August 2013	Rivoli Bay, Beachport, SA
Phacelocarpus sp.	27th August 2013	Rivoli Bay, Beachport, SA
Phyllospora comosa	27th August 2013	Rivoli Bay, Beachport, SA

Sargassum sp.	27th August 2013	Rivoli Bay, Beachport, SA
Sargassum axillaris	27th August 2013	Rivoli Bay, Beachport, SA
Seirococcus axillaris	27th August 2013	Rivoli Bay, Beachport, SA

Figure 2.1 Photograph of Rivoli Bay, Beachport SA was taken at 27 August, 2013

2.2 Extraction Method of Phlorotannins

The polyphenolic compounds from the selected Australian native seaweeds were extracted using an optimized protocol from the laboratory (Charoensiddhi, 2014). Dried seaweeds were ground and homogenized to fine powder by blender and then passed through a 0.25-mm sieve to get uniform powder. The 0.5g of seaweed powder was extracted with 5ml of solvent (90% ethanol) and

incubated in shaker at 200 rpm for 3 hours. Extracted sample centrifuged at 5500 rpm for 20 minutes at 4^0 C. For solvent extraction, the collected supernatant is evaporated in rotary evaporator for 5-6 hours at room temperature to evaporate the ethanol. The extract obtained after evaporated organic solvent (90% ethanol) placed in -80^0 C for 2 hours then placed in freeze drier for one day. After freeze drying stored the crude extract at -20^0 C for further analysis.

2.3 Phlorotannins extraction from *Ecklonia radiata* (10g) to Scale up the production

Seaweeds of five different species showed high total phenolics content and antioxidant activities. Out of those seaweed species *Ecklonia radiata* extracted in large scale due to its significant bioactive properties. *Ecklonia radiata* is one of the most abundant brown seaweed species in Southern Australia, which produces largest fraction of biomass and examined for bioactive compounds.

For analysis, 10gm dried blended seaweed powder of *Ecklonia radiata* was extracted with 100 ml of solvent (90% ethanol) and incubated in shaker at 200 rpm for 3 hours. Extracted sample centrifuged at 5500 rpm for 20 minutes at 4^0 C. For solvent extraction, the collected supernatant is evaporated in rotary evaporator for 8-10hrs at room temperature to evaporate the ethanol. The extract obtained after evaporated organic solvent (90% ethanol) placed in -80^0 C for 2 hours then placed in freeze drier for one day. After freeze drying stored the crude extract at -20^0 C for further analysis.

2.4 Total phenolics content analysis with Mili-Q water

For determination of total phenolic content (TPC) and antioxidant activities, 10mg freeze dried powder of seaweeds mixed with 1ml of Mili-Q water for sample preparation. The prepared samples further tried to dilute 10 times, 20 times and 40 times for each seaweed extract to obtain absorbance value 0.2 to 0.4 which was proven to be accurate for standard curve values in the TPC and antioxidant assays. For 10 times dilution 10ul sample diluted with 90ul of Mili-Q water, for 20 times dilution 5ul sample diluted with 95ul of Mili-Q water and for 40 times dilution 5ul sample diluted with 195ul of Mili-Q water.

Total phenolics (TP) assay was used to determine the total phenolics content in the Australian native seaweeds. Presence of phenolics compounds in the seaweeds was determined using Folin-Ciocalteu phenol reagent which was adapted from of Koivikko et al. (2005) and Wang et al. (2012) with and modified in our laboratory. In this assay, 100μl of diluted prepared samples of seaweeds with MiliQ water mixed with 500μl of Folin-Ciocalteu reagent (10% in Mili-Q water) and vortex the eppendorf tubes. After 5 minutes, 400μl of sodium carbonate (7.5% in Mili-Qwater) added and mixed completely. Then the samples were incubated for 2 hours in the dark. After 2 hours incubation, the absorbance was measured at 725nm with a micro-plate reader (μQuant, Biotek Instruments, Inc., USA). A standard curve with serial dilutions of phloroglucinol (20-100μg/ml) with Mili-Q water was used for calibration. The results are expressed in grams of phloroglucinol equivalents (PGEs) per 100g of dry weight (DW) of seaweed. The following formulas were used to determine the total phenolics content in the dried seaweeds.

Equation 2.1

Concentration of total phenolics (μg PGEs/ ml extract)

= (Absorbance at 725nm/ slope) × Dilution factor

Equation 2.2

Total phenolics content (gm PGEs/ 100g DW of seaweed)

= **{**(concentration of total phenolics × volume of Mili-Q water used (L) / weight of freeze dried sample used (gm)**}** × yield (weight of total freeze dried seaweed (gm)) / weight of dry seaweed used for extraction (gm)

Equation 2.3

Total phenolics content (gm PGEs/100g of extract)

= Total phenolics content (gm PGEs/ 100g DW of seaweed) / (yield/100g DW of seaweed) × 100

2.5 Antioxidant assays with Mili-Q water

As per previous research in literature various antioxidant assays used to determine the antioxidant activity from seaweeds. Most common of them are hydrogen atom transfer reactions such as Oxygen Radical Absorbance Capacity (ORAC) assay and Ferric reducing antioxidant power (FRAP) and in this study these two assays were used to determine the antioxidant activities.

2.5.1 Ferric reducing antioxidant power (FRAP) assay with Mili-Q water

FRAP assay measures the total antioxidant activity based on the total reducing capacity of electron-donating antioxidants. It is based on the ability of antioxidant power to reduce (electron transfer) Fe^{3+} to Fe^{2+} ions in the presence of tripyridyltriazine (TPTZ) forming an intense blue Fe^{2+} - TPTZ complex. In previous studies, FRAP assay (Benzie & Strain, 1996) and this method modified in our laboratory as safe operating procedure (SOP) by Suvimol Charoensiddi in 2014. In this assay, seaweed extract was centrifuged for 10 minutes at 7500 rpm prior to use and the supernatant was saved. Then the extract was diluted with Mili-Q water using appropriate dilution factor. In brief,

FRAP reagent contain 25ml of 300 mM acetate buffer at pH 3.6, 2.5ml 10 mM TPTZ, and 2.5ml of 20 mM $FeCl_3.6H_2O$. 300μl of FRAP reagent pipette into eppendorf tube and treated with 37^0C in heating bath for 10 minutes. Then 10μl of samples and 30μl of Mili-Q water added to the FRAP reagent and mixed well. The samples incubate for another 4 minutes and then measure the absorbance at 593nm in micro-plate reader (μQuant, Biotek Instruments, Inc., USA) against a blank consisted of 10μl standard sample of $FeSO_4.7H2O$ with standard concentration (0.1 to 1.0mM) with miliQ water, 300μl FRAP reagent and 30μl of Mili-Q water. The adequate absorbance reading for each sample is between 0.2 and 0.4 for a reliable calculation. The antioxidant activity of the tested seaweeds is expressed as mmol $FeSO_4$ equivalents / 100g DW of seaweed and calculated by the following equations:

Equation 2.4

FRAP value (mM $FeSO_4$ equivalents/ 100g DW of seaweed)

= {(Absorbance at 593nm / slope) × dilution factor} × (yield (mg) / weight of freeze dried sample used (μg)) / weight of dry seaweed powder used (eg.0.5g) × 100

Equation 2.5

FRAP value (mM $FeSO_4$ equivalents / 100g TPC)

= FRAP value (mM $FeSO_4$ equivalents / 100g DW of seaweed) / Total phenolics content (gm PGEs/ 100g DW of seaweed) × 100

Equation 2.6

FRAP value (mM $FeSO_4$ equivalents / 100g extract)

= FRAP value (mM $FeSO_4$ equivalents / 100g DW of seaweed) / yield / 100g seaweed × 100

2.5.2 Oxygen Radical Absorbance Capacity (ORAC) assay with Mili-Q water

ORAC assay measures the antioxidant scavenging activity against peroxyl radical induced by 2,2′-azobis(2methylpropionamidine) dihydrochloride (AAPH). The ORAC assay was performed according to Huang et al. (2002) and Wang et al. (2009) with slight modifications and optimized in our laboratory. AAPH (0.414 g) was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) at a final concentration of 153 mM and then kept in an ice bath. Fluorescein stock solution (4.19×10−3 mM) was prepared in 75mMphosphate buffer (pH 7.4) and was kept at 4 °C in dark condition. The 8.16×10−5 mM fresh fluorescein working solution was made freshly at the time of experiment by further diluting the stock solution in 75 mM phosphate buffer (pH 7.4).

Microplate reader DTX 880 (Beckman Coulter Inc., USA) was used for the fluorescence measurements. Trolox (standard) and seaweeds extract were diluted in 96-well plate. Trolox was prepared in concentrations of 100µM, 75µM, 50µM, 25µM, 12.5µM AND 0µM while the dilution factors of 10x, 20x, 50x, 100x, 200x, 400x, 800x, 1600x and 3200x were applied for each seaweed extract. Three lowest dilutions factors (50x, 200x and 400x) normally used in the analysis. During analysis, 180µl of fluorescein working buffer, 30 µl of phosphate working buffer and 30µl of diluted sample/Trolox loaded into each well. The analysis plate was pre-incubated at 37 °C for 10 min before adding the AAPH. The reaction is initiated by the addition of 30 µL of 153 mM AAPH solution. The fluorescence reading (f_0) was recorded at 0 min then every 1.5 min ($f_1, f_2 f_3 \ldots\ldots$) for 50 cycles. Two different excitation and emission filter wavelengths were set at 485 and 535 nm, respectively to measure the absorbance. The final ORAC value was calculated by using a regression equation between Trolox concentration and net area under curve (AUC) and performed by MATLAB program by using an equation 2.7

Equation 2.7

AUC = 0.5 + f_i/f_0 +…..+ f_{34}/f_0 +0.5 x (f_{50}/f_0)

Where f_0 = Initial fluorescence reading at 0 min, f_1 = fluorescence reading at time i. The net AUC was obtained by subtracting the AUC of the blank from that of a sample. Net AUC value between 15 and 20 was desirable for a reliable measurement. The calculation of AUC expressed as μmol Trolox equivalents (TE)/100g DW of seaweed using the calibration curve of Trolox standards (concentration 0–100 μM) and as shown in formula below.

Equation 2.8

ORAC value (μM Trolox equivalents (TE))

= {(Net AUC / slope) × dilution factor}

Equation 2.9

ORAC value (μM Trolox equivalents/ 100g DW of seaweed)

ORAC value (μmol TE/100g DW of extract) /100 × (yield / 100 g DW of seaweed)

Equation 3.0

ORAC value (μM Trolox equivalents / 100g TPC)

= ORAC value (μM $FeSO_4$ equivalents / 100g DW of seaweed) / Total phenolics content (gm PGEs/ 100g DW of seaweed) × 100

2.6 Total phenolics content analysis with 90% ethanol

10mg freeze dried extract of seaweeds dissolved with 1ml of 90% ethanol for sample preparation. The prepared samples further tried to dilute 10 times, 20 times and 40 times for each seaweed extract with 90% ethanol to obtain absorbance value 0.2 to 0.4 which was proven to be accurate for standard curve values in the TPC and antioxidant assays. For 10 times dilution 10ul sample diluted with 90ul of 90% ethanol, for 20 times dilution 5ul sample diluted with 95ul of 90% ethanol and for 40 times dilution 5ul sample diluted with 195ul of 90% ethanol.

In this assay, 100μl of diluted prepared samples of seaweeds with 90% ethanol mixed with 500μl of Folin-Ciocalteu reagent (10% in Mili-Q water) and vortex the eppendorf tubes. After 5 minutes, 400μl of sodium carbonate (7.5% in Mili-Qwater) added and mixed completely. Then the samples were incubated for 2 hours in the dark. After 2 hours incubation, the absorbance was measured at 725 nm with a micro-plate reader (μQuant, Biotek Instruments, Inc., USA). A standard curve with serial dilutions of phloroglucinol (20-100μg/ml) with 90% ethanol was used for calibration. The results are expressed in grams of phloroglucinol equivalents (PGEs) per 100g of dry weight (DW) of seaweed. The following formulas were used to determine the total phenolics content in the dried seaweeds.

Equation 2.1

Concentration of total phenolics (μg PGEs/ ml extract)

= (Absorbance at 725nm/ slope) × Dilution factor

Equation 2.2

Total phenolics content (gm PGEs/ 100g DW of seaweed)

= **{**(concentration of total phenolics × volume of Mili-Q water used (L) / weight of freeze dried sample used (gm)**}** × yield (weight of total freeze dried seaweed (gm)) / weight of dry seaweed used for extraction (gm)

Equation 2.3

Total phenolics content (gm PGEs/100g of extract)

= Total phenolics content (gm PGEs/ 100g DW of seaweed) / (yield/100g DW of seaweed) × 100

2.7 Ferric reducing antioxidant power (FRAP) assay with 90% ethanol

In this assay, seaweed extract was centrifuged for 10 minutes at 7500 rpm prior to use and the supernatant was saved. Then the extract was diluted with 90% ethanol using appropriate dilution factor. FRAP reagent contain 25ml of 300 mM acetate buffer at pH 3.6, 2.5ml 10 mM TPTZ, and 2.5ml of 20 mM $FeCl_3.6H_2O$. 300μl of FRAP reagent pipette into eppendorf tube and treated with 37^0C in heating bath for 10 minutes. Then 10μl of samples and 30μl of 90% ethanol added to the FRAP reagent and mixed well.

The samples incubate for another 4 minutes and then measure the absorbance at 593nm in micro-plate reader (μQuant, Biotek Instruments, Inc., USA) against a blank consisted of 10μl standard sample of $FeSO_4.7H2O$ with standard concentration (0.1 to 1.0mM) with 90% ethanol, 300μl FRAP reagent and 30μl of 90% ethanol. The adequate absorbance reading for each sample is between 0.2 and 0.4 for a reliable calculation. The antioxidant activity of the tested seaweeds is expressed as mmol $FeSO_4$ equivalents / 100g DW of seaweed and calculated by the following equations:

Equation 2.4

FRAP value (mM $FeSO_4$ equivalents/ 100g DW of seaweed)

= {(Absorbance at 593nm / slope) × dilution factor} × (yield (mg) / weight of freeze dried sample used (μg)) / weight of dry seaweed powder used (eg.0.5g) × 100

Equation 2.5

FRAP value (mM $FeSO_4$ equivalents / 100g TPC)

= FRAP value (mM $FeSO_4$ equivalents / 100g DW of seaweed) / Total phenolics content (gm PGEs/ 100g DW of seaweed) × 100

Equation 2.6

FRAP value (mM $FeSO_4$ equivalents / 100g extract)

= FRAP value (mM $FeSO_4$ equivalents / 100g DW of seaweed) / yield / 100g seaweed × 100

2.8 Oxygen Radical Absorbance Capacity (ORAC) assay with 90% ethanol

In this assay, sample prepared with 90% ethanol used for analysis after 10 min centrifugation at 7500rpm. During analysis, AAPH (0.414 g) was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) at a final concentration of 153 mM and then kept in an ice bath. Fluorescein stock solution (4.19×10^{-3} mM) was prepared in 75mMphosphate buffer (pH 7.4) and was kept at 4 °C in dark condition. The 8.16×10^{-5} mM fresh fluorescein working solution was made freshly at the time of experiment by further diluting the stock solution in 75 mM phosphate buffer (pH 7.4).

Microplate reader DTX 880 (Beckman Coulter Inc., USA) was used for the fluorescence measurements. Trolox (standard) and seaweeds extract were diluted in 96-well plate. Trolox was prepared in concentrations of 100µM, 75µM, 50µM, 25µM, 12.5µM AND 0µM while the dilution factors of 10x, 20x, 50x, 100x, 200x, 400x, 800x, 1600x and 3200x were applied for each seaweed extract. Three lowest dilutions factors (50x, 200x and 400x) normally used in the analysis. During analysis, 180µl of fluorescein working buffer, 30 µl of phosphate working buffer and 30µl of

diluted sample/Trolox loaded into each well. The analysis plate was pre-incubated at 37 °C for 10 min before adding the AAPH. The reaction is initiated by the addition of 30 µL of 153 mM AAPH solution. The fluorescence reading (f_0) was recorded at 0 min then every 1.5 min ($f_1, f_2 f_3$……) for 50 cycles. Two different excitation and emission filter wavelengths were set at 485 and 535 nm, respectively to measure the absorbance. The final ORAC value was calculated by using a regression equation between Trolox concentration and net area under curve (AUC) and performed by MATLAB program by using an equation 2.7.

Equation 2.7

$$AUC = 0.5 + f_i/f_0 + \ldots.. + f_{34}/f_0 + 0.5 \times (f_{50}/f_0)$$

Where f_0 = Initial fluorescence reading at 0 min, f_1 = fluorescence reading at time i. The net AUC was obtained by subtracting the AUC of the blank from that of a sample. The calculation of AUC expressed as µmol Trolox equivalents (TE)/100g DW of seaweed using the calibration curve of Trolox standards (concentration 0–100 µM) and as shown in formula below.

Equation 2.8

ORAC value (µM Trolox equivalents (TE))
= {(Net AUC / slope) × dilution factor}

Equation 2.9

ORAC value (µM Trolox equivalents/ 100g DW of seaweed)
ORAC value (µmol TE/100g DW of extract) /100 × (yield / 100 g DW of seaweed)

Equation 3.0

ORAC value (μM Trolox equivalents / 100g TPC)

= ORAC value (μM $FeSO_4$ equivalents / 100g DW of seaweed) / Total phenolics content (gm PGEs/ 100g DW of seaweed) × 100

2.9 Statistical analysis

For the statistical analysis, One-way Anova was used to compare the means in selected samples in duplicate during total phenolics analysis to establish reproducibility, and used peripheral standards to verify that the results were accurate. Ferric reducing antioxidant capacity (FRAP) assay and oxygen radical absorbance capacity (ORAC) assay was conducted in triplicate, with data presented as mean values ± standard deviation. One-way analysis of variance (ANOVA) was used to compare the variations within TPC and antioxidant activities were considered significant at $p<0.05$.

Chapter 3: Results

3.1 Extraction yield

The conventional extraction method with 90% ethanol used for extraction which gave nearly 5-20% yield (Dry weight of freeze dried extract) after extraction from the selected seaweed species. In which some seaweed species give high yield such as *Curdica angustata* (21.38%) yield /100g DW of seaweed > *Cystophora platylobium* (16.6%) / 100g DW of seaweed > *Ecklonia radiata* (13.6%) /100g DW of seaweed > *Macrocystis angustifolia* (14.2% / 100g DW of seaweed > *Phyllospora comosa* and *Sargassum sp.* both (11%) / 100g DW of seaweed respectively. The detailed illustration of yield data for all seaweed species is shown in Table 3.1. In all tables and graphs [R] represents red seaweed, [G] represents green seaweed, no symbol represents brown seaweeds.

Table 3.1 Extraction yield by 90% ethanol of selected seventeen South Australian native seaweeds

Seaweeds	**Yield /100g DSW**
Callophyllis lambertii [R]	4.74
Melanthalia obustata [R]	4.1
Phacelocarpus sp. [R]	7.6
Curdica angustata [R]	21.38
Codium galeatum [G]	9.4
Carpoglossum confluens	8.8
Cystophora monilifera	4.86
Cystophora moniliformis	6.2
Cystophora platylobium	16.6
Durvillaea potatorum	4.6
Ecklonia radiata	13.6
Macrocystis angustifolia	14.2

Macrocystis pyrifera	5
Phyllospora comosa	11
Sargassum sp.	11
Sargassum axillaris	5.04
Sierococcus axillaris	7.8

3.2 Yield of total phenolics content

From the results, the total phenolics content varied widely in the seventeen Australian native seaweed species, which ranged from 0.3 to 5.4g PGEs /100g DW of seaweed with Mili-Q water and 0.2 to 5.5g PGEs /100g DW of seaweed with 90% ethanol . Some of the seaweeds give high total phenolics content with decreasing total phenolics content tested with Mili-Q water in order were *Ecklonia radiata* (5.4g PGEs /100g DW of seaweed) > *Ecklonia radiata* (10g) (4.3g PGEs /100g DW of seaweed) > *Sargassum sp*. (2.9g PGEs /100g DW of seaweed) > Cystophora monilifera (1.9g PGEs /100g DW of seaweed) > Cystophora platylobium (1.7 PGEs /100g DW of seaweed) *Carpoglossum confluens* (1.01g PGEs /100g DW of seaweed).

On the other hand, seaweeds showed high total phenolics content with 90% ethanol with decreasing total phenolics content in order were *Ecklonia radiata* (5.5g PGEs /100g DW of seaweed) > *Ecklonia radiata* (10g) (4.5g PGEs /100g DW of seaweed) > Cystophora platylobium (3.8 PGEs /100g DW of seaweed) > *Sargassum sp*. (3.3g PGEs /100g DW of seaweed) > Cystophora monilifera (1.8g PGEs /100g DW of seaweed) > *Carpoglossum confluens* (1.01g PGEs /100g DW of seaweed). Moreover, seaweed species *Cystophora moniliformis, Durvillaea potatorum, Macrocystis angustifolia, Phyllospora comosa, Macrocystis pyrifera* and *Sargassum axillaris* give moderate total phenolics content which ranged from 0.1 to 0.4g PGEs/100g DW of seaweeds. The

seaweed species those possessed low phenolics content were *Sierococcus axillaris*, *Phacelocarpus sp.*, *Callophyllis lambertii*, *Codium galeatum* and *Melanthalia obustata* which ranged from 0.03 to 0.08g PGEs /100g DSW(Dry seaweed weight).

Figure 3.1: Yield of total phenolics content in phloroglucinol gram equivalents / 100g DSW of selected seventeen South Australian native seaweeds

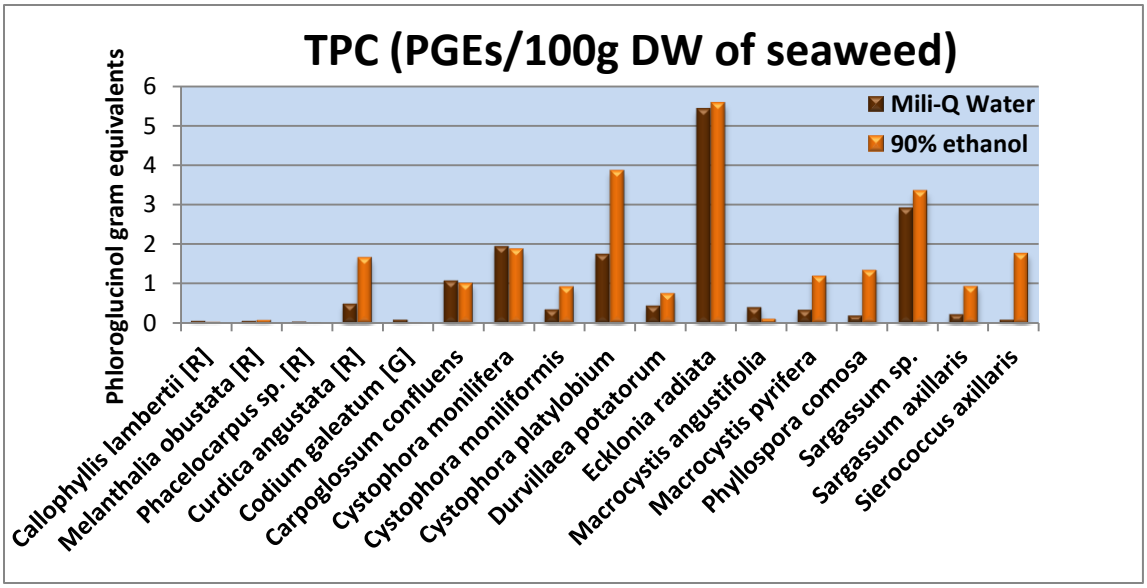
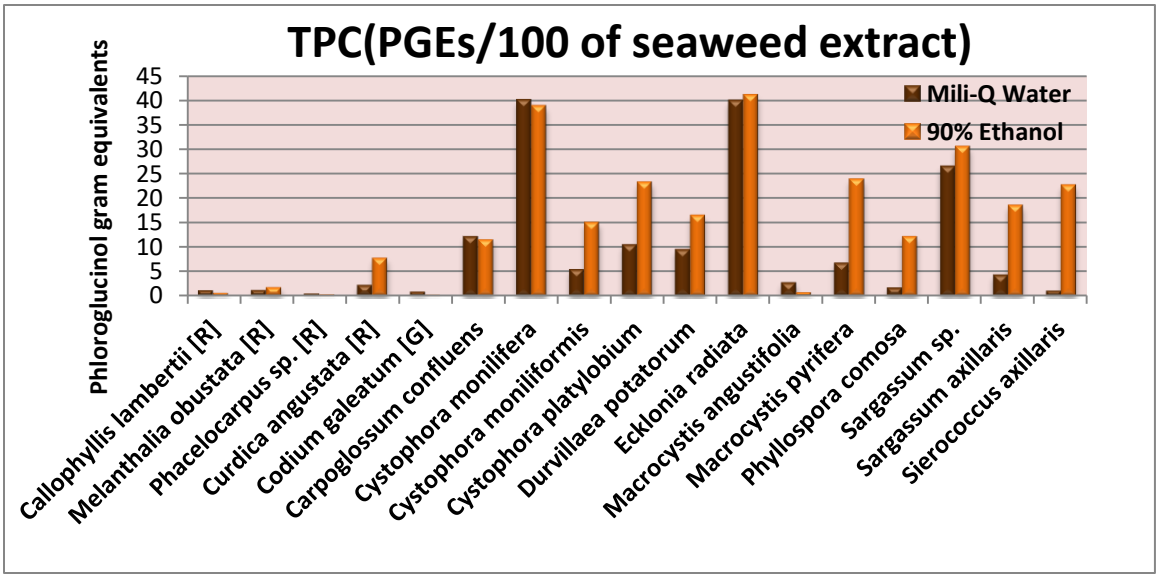


Figure 3.2: Yield of total phenolics content in phloroglucinol gram equivalents / 100g of seaweed extract of selected seventeen South Australian native seaweeds



3.3 Antioxidant properties of selected seventeen South Australian native seaweeds

In this study, the antioxidant activities of the selected Australian native seaweeds were measured using two common antioxidant assays, FRAP assay and ORAC assay. These two assays measured antioxidant activity based on different principles. FRAP assay measures the total antioxidant activity based on the total reducing capacity of electron donating antioxidants (Benzie & Strain 1996). ORAC assay measures the effectiveness of the antioxidant sample which reflects the antioxidant activity of a particular sample against peroxyl radicals (Huang et al., 2002).

3.3.1 Ferric reducing antioxidant activity

The result of the ferric reducing antioxidant properties of the selected seventeen Australian native seaweeds are in Figure 3.3. It showed that there is significant difference ($p<0.05$) in antioxidant properties ranged from 0.09 to 29.5 mmol FE/100g DSW (Mili-Q water) and 1.4 to 57.5mmol FE/100g DSW (90% Ethanol) of the selected seventeen seaweed species. The lowest to moderate antioxidant activities ranged from (0.09 to 6.0 mmol FE (FeSO4 eq.) /100g DSW) (Dry seaweed weight) for given seaweed species which tested with Mili-Q water (0.1 to 20.0 mmol FE /100g DSW) for these seaweeds when tested with 90% ethanol are as *Callophyllis lambertii, Codium galeatum, Curdica angustata, Cystophora platylobium, Durvillaea potatorum, Macrocystis angustifolia, Macrocystis pyrifera, Melanthalia obustata, Phacelocarpus sp, Phyllospora comosa, Sargassum axillaris, Sierococcus axillaris* and *Carpoglossum confluens.*

The antioxidant activities showed 5-10% raise when they tested with 90% ethanol. Some of the seaweed species showed high antioxidant activities when tested with Mili-Q water called *Ecklonia radiata* (28.3 mmol FE /100g DSW), *Ecklonia radiata* (10g) (29.7 mmol FE / 100g DSW) but when *E.radiata* tested with 90% ethanol it nearly doubles the activity of *E.radiata* (57.1 mmol FE/ 100g

DSW) and for *E.radiata* (10g) gave (48.3 mmol FE /100g DSW) antioxidant activity. After that, *Cystophora monilifera* gave high antioxidant activity (13.5 mmol FE/ 100g DSW) with MIli-Q water but with 90% ethanol it showed (18.6 mmol FE /100g DSW).

It was observed in general that some of the seaweeds have high phenolics content and high antioxidant activity in FRAP assay (Figure 3.3, 3.4, 3.5). Although, most seaweeds gave low to moderate antioxidant activity but high phenolics content observed in most of seaweed species those tested with Mili-Q water e.g. *C.galeatum* (1968.1 mmol FE /100g TPC), *Phacelocarpus sp*. (2348.1mmol FE /100g TPC) and *Sierococcus axillaris* (2238.1 mmol FE /100g TPC). On the other hand, those seaweed species show high antioxidant activity those gave moderate total phenolics content e.g. *E.radiata* (521.1mmol FE/100g TPC) with mili-Q water and (1403.1 mmol FE /100g TPC) with 90% ethanol.

Figure 3.3: Antioxidant activity by FRAP assay in mmol FeSO4 eq./100g DSW of selected seventeen South Australian native seaweeds

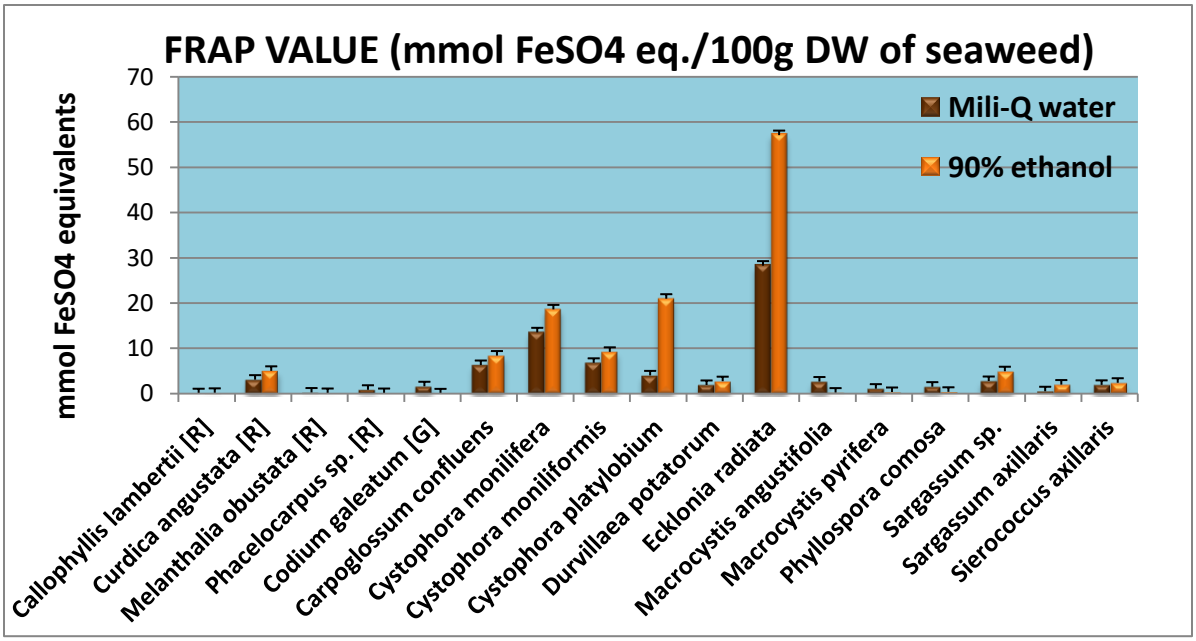


Figure 3.4: Antioxidant activity by FRAP assay in mmol FeSO4 eq./100g of seaweed extract of selected seventeen South Australian native seaweeds

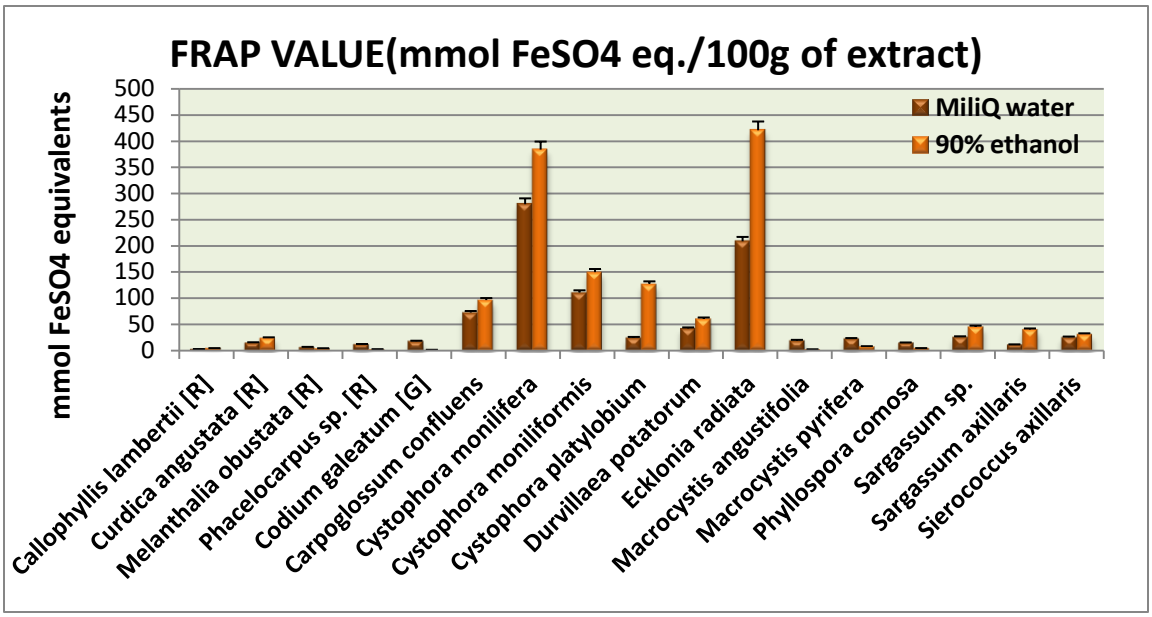
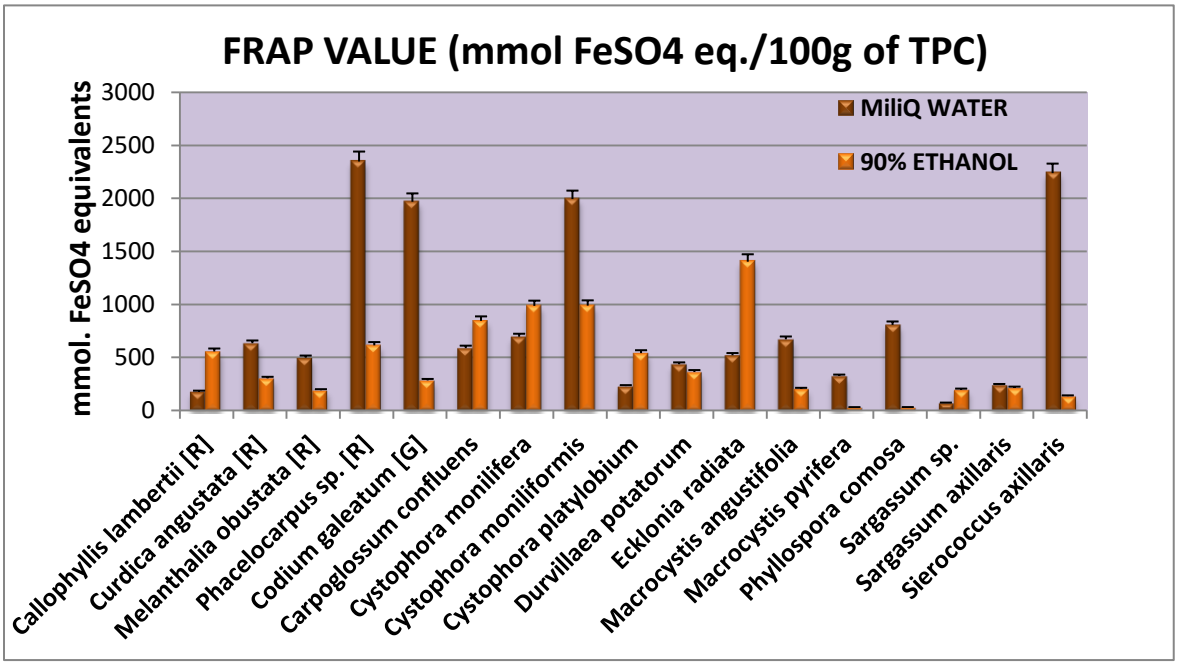


Figure 3.5: Antioxidant activity by FRAP assay in mmol FeSO4 eq./100g of TPC of selected seventeen South Australian native seaweeds



3.3.2 Oxygen radical absorbance antioxidant activity

Antioxidant activities by oxygen radical absorbance capacity assay ranged from 0.3 to 795 µmol TE/ 100g DSW (Mili-Q water), 96.7 to 820.5 µmol TE/ 100g DSW (90% ethanol) for all seventeen seaweed species. Very low antioxidant activities observed in some seaweed species with Mili-Q water (0.3 to 63.5 µmol TE/ 100g DSW) but antioxidant value increased to 5-10% with 90% ethanol (80.5 to 545.5 µmol TE/ 100g DSW) for the same seaweed species. These seaweed species include *C.lambertii, C.angustata, C.confluens, C.angustata, C.platylobium, D.potatorum, M.angustifolia, M.pyrifera, Phacelocarpus sp.* and *S.axillaris.*

Only few seaweed species showed high antioxidant activity with Mili-Q water whereas most of seaweeds gave high antioxidant with 90% ethanol. Some water tested seaweeds did not show any activity but after testing with 90% ethanol they showed good antioxidant activity. *Ecklonia radiata, Sargassum sp.* and *C.monilifera* showed high antioxidant activities with both Mili-Q water and 90% ethanol which ranged from 623.7 to 794.1 µmol TE/ 100g DSW. The 90% ethanol tested seaweeds also gave high (7779.1 to 256746.2 µmol TE/ 100g TPC) phenolics content for most of seaweeds whereas water tested seaweeds give low (156.2 to 93301.9 µmol TE/ 100g TPC) total phenolics content and antioxidant activities.

On the other hand, totally different profile was observed for antioxidant activities /100g of seaweed extract. Most of seaweeds were analyzed with 90% ethanol showed good antioxidant activities (2000 to 6000 µmol TE/ 100g of seaweed extract). But four seaweeds include *Cystophora monilifera* > *Sargassum sp.* > *Ecklonia radiata* > and *Macrocystis pyrifera* gave high antioxidant activities/ 100g seaweed extract. Otherwise, other seaweeds gave low antioxidant activities with Mili-Q water but medium to higher/100g seaweed extract with 90% ethanol.

Figure 3.6 Antioxidant activities by ORAC assay in µmol Trolox equivalents /100g DSW of selected seventeen South Australian native seaweeds

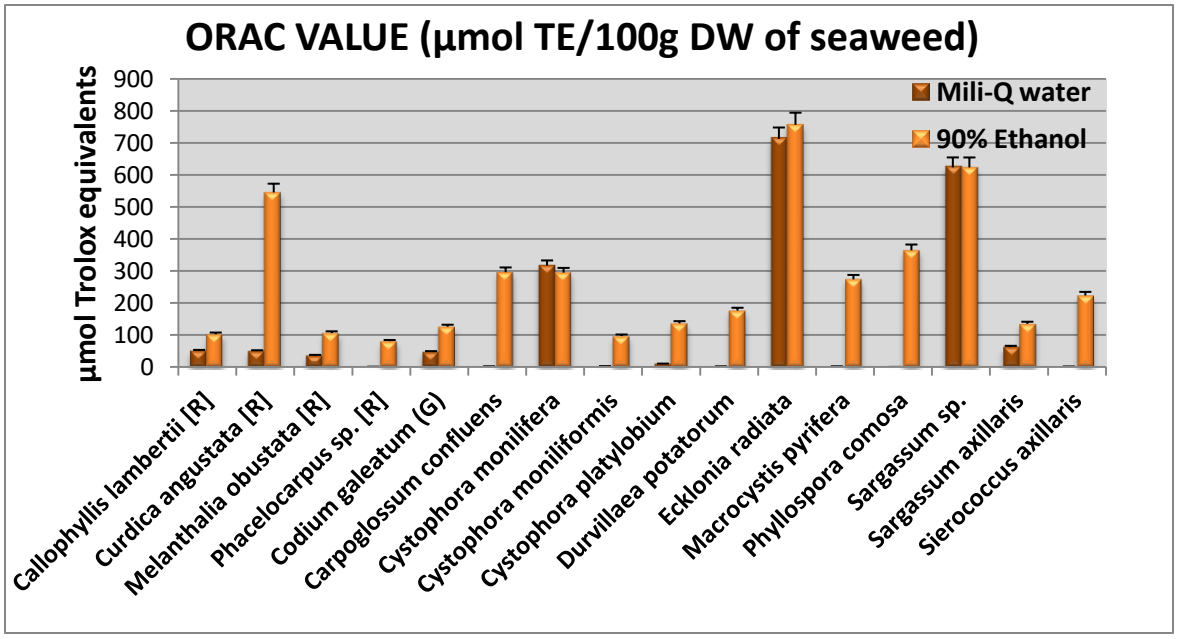


Figure 3.7 Antioxidant activities by ORAC assay in µmol Trolox equivalents /100g of seaweed extract of selected seventeen South Australian native seaweeds

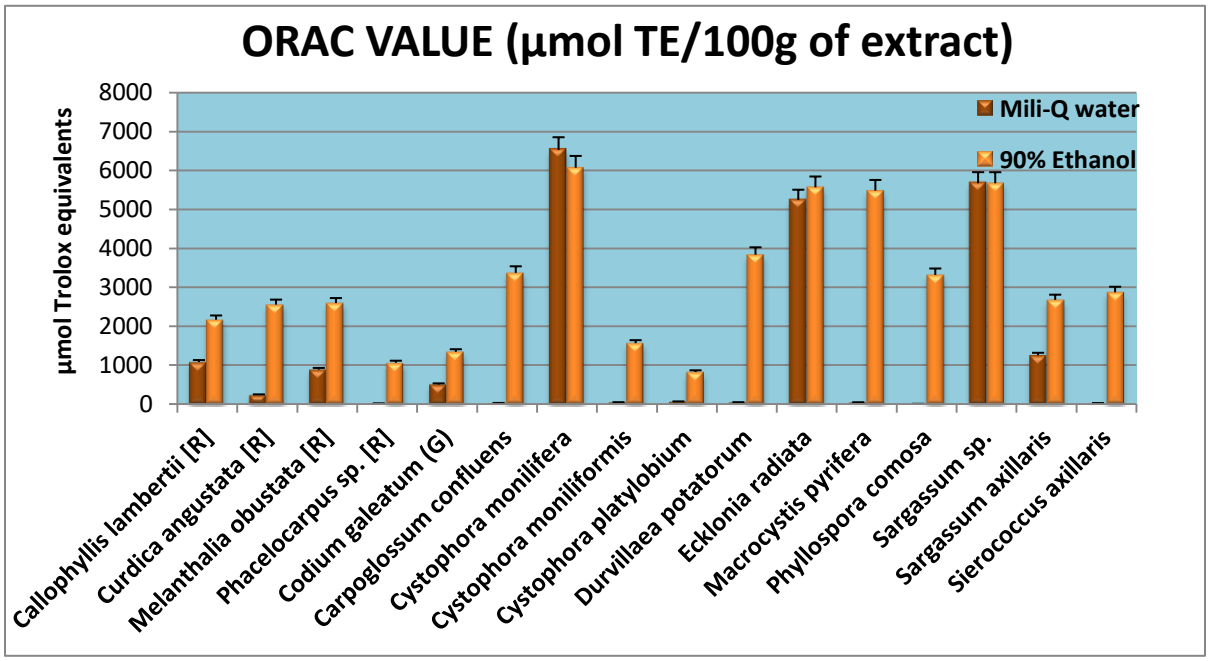
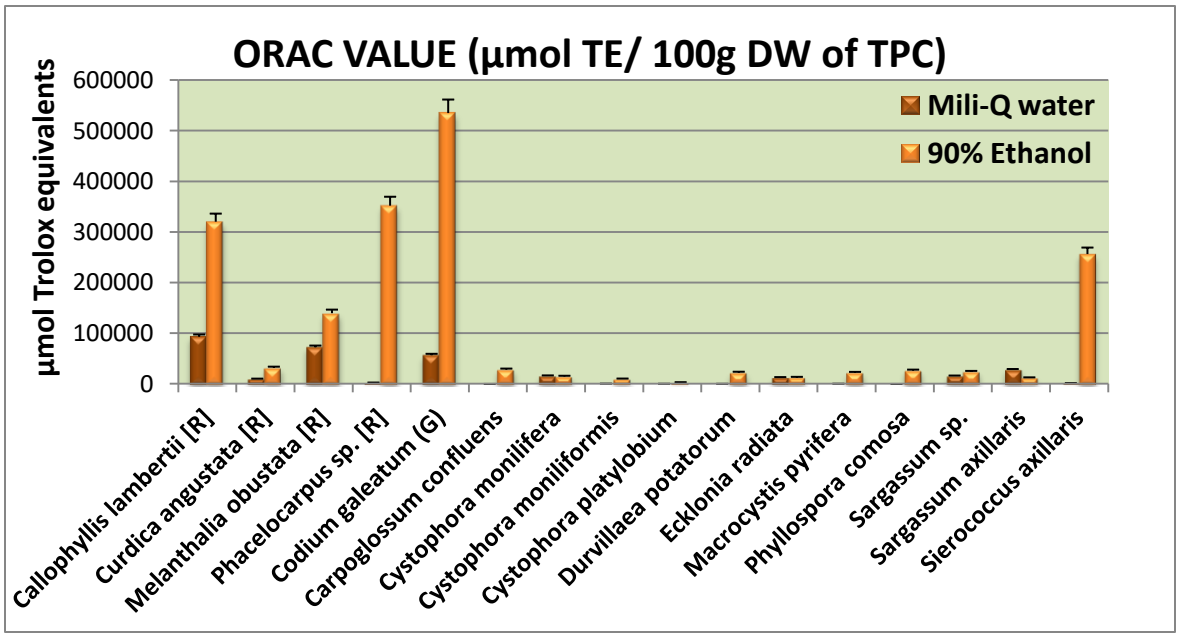


Figure 3.8 Antioxidant activities by ORAC assay in µmol Trolox equivalents /100g of TPC of selected seventeen South Australian native seaweeds



3.4 Total phenolics content and antioxidant activities of *Ecklonia radiata* (10g) at large scale

Due to high TP content and good antioxidant activities of *Ecklonia radiata* it was chosen for large scale production. According to results of extraction yield, total phenolics content and antioxidants activities by FRAP and ORAC assay of 0.5g of *Ecklonia radiata*. 10g of *Ecklonia radiata* is used for large scale productio which gave nearly same total phenolics content [(4.3g PGEs/100g DSW with Mili-Q water), (4.5g PGEs/100g DSW with 90% ethanol)] and antioxidant activities [(FRAP 29.7mmol FE/100g DSW with MQ water), (FRAP 48.5 mmol FE/100g DSW with 90% ethanol)] and [(ORAC 794.1µmol TE/100g DSW with MQ water), (ORAC 820.1µmol TE/100g DSW with 90% ethanol)].

Chapter 4: Discussion

4.1 Extraction Yield of selected seventeen South Australian native seaweeds

Extraction yield ranged from 5-20% from all the selected seventeen South Australian native seaweed species. In which some seaweed species give high yield such as *Curdica angustata* (21.38%) yield /100g DW of seaweed > *Cystophora platylobium* (16.6%) / 100g DW of seaweed > *Ecklonia radiata* (13.6%) /100g DW of seaweed > *Macrocystis angustifolia* (14.2% / 100g DW of seaweed > *Phyllospora comosa* and *Sargassum sp.* both (11%) / 100g DW of seaweed respectively. On the other hand, seaweeds including *C.lambertii, D.potatorum, M.obsutata, M.pyrifera* showed very low extraction yield from 4.5 to 6% /100g DSW. Otherwise, other seaweeds gave medium yield and significant differences were observed in yield of species belongs to same genera such as *Cystophora* species, *Macrocystis* species and *Sargassum* species (Table 3.1).

4.2 Great variations in total phenolics content of selected seventeen South Australian native seaweeds

As research point of view polyphenols was increasingly used in recent years as protective dietary constituents in plants including fruits and vegetables. In addition, research of plant polyphenols for industrial applications, such as medical, pharmaceutical, nutraceutical, cosmetic and cosmeceutical are being important plant derived bioactives are generally regarded as low cost and less toxic (Pandey et al., 2009). According to previous research in literature review seaweeds are the rich sources of antioxidant compounds and polyphenols are one of them. Seaweed polyphenols show wide range of activities namely antioxidant (Sathya et al.), anti-inflammatory (Liu et al., 2014), anti-allergic (Barbosa et al., 2014b), antimicrobial (Eom et al., 2012), anticancer (Kim et al., 2011) and antidiabetic (Lee and Jeon, 2013) activities. As a result, this study has focused on the screening of

polyphenols for antioxidant activities of selected seventeen South Australian native seaweeds. Great variations were found in total phenolics content of seventeen different species of seaweeds which varied from 0.3 to 5.4g PGEs /100g DW of seaweed with Mili-Q water and 0.2 to 5.5g PGEs /100g DW of seaweed with 90% ethanol . Five genera of seaweeds gave high phenolics content which ranged from 1.01 to 5.4g PGEs /100g DW of seaweed with mili-Q water and 1.7 to 5.5g PGEs /100g DW of seaweed with 90% ethanol. They were *Ecklonia radiata* > *Sargassum sp*. > Cystophora monilifera > Cystophora platylobium > *Carpoglossum confluens.*

It was also found that chemical profiles change greatly among different species within the same genus. In this research three different species of *Cystophora* (*C.platylobium* 1.75g PGEs/100gDSW), (*C.monilifera* 1.94g PGEs/100gDSW), (*C.moniliformis* 0.34g PGEs/100gDSW), two different species of *Macrocystis* (*M.pyrifera* 0.34 PGEs/100gDSW), (*M.angustifolia* 0.39g PGEs/100gDSW) and two different species of *Sargassum* (*Sargassum sp*.2.9g PGEs/100gDSW), (*S. axillaris* 0.2g PGEs/100gDSW) has been studied and all give different profiles of total phenolics content (Table 3.2) and antioxidant activities (Table 3.3 & 3.4) although they belongs to same genus.

There is significant difference was found in phenolic content ($p < 0.05$) for all seaweed species. In general, high total phenolics content obtained by 90% ethanol tested seaweed species comparatively Mili-Q water. The difference in results of total phenolics content due to solubility factor because according to results seaweed extracts are not water soluble and they dissolved completely in solvent (90% ethanol) because they extracted by 90% ethanol conventional method.

4.3 Significant differences in antioxidant activities by FRAP and ORAC assays

Two antioxidant assays, FRAP and ORAC were performed to measure the antioxidant activity of the selected seventeen seaweed species. In the SET based assay (FRAP assay), antioxidants are oxidized by oxidants Fe(III) and leading to single electron transfer from the antioxidant molecule to the oxidant, which changed the colour while reduction (Ou et.al, 2002). It is sensitive, simple, rapid, highly reproducible and inexpensive method. ORAC assay is HAT based assay which utilizes a radical initiator to generate peroxyl radicals, in which antioxidants and substrate compete for thermally generated peroxyl radicals (Ou et. al, 2002). This assay is highly specific and responds to numerous antioxidants. In this study, free radical scavenging capacities was determined from the selected seventeen South Australian native seaweeds using ORAC assay and ferric reducing capacities using FRAP assay.

As shown in the Figure 3.3, huge variations found in both FRAP values [0.09 to 29.5 mmol FE/100g DSW (Mili-Q water)], [1.4 to 57.5mmol FE/100g DSW (90% Ethanol)] and ORAC values from [0.3 to 795 µmol TE/ 100g DSW (Mili-Q water)], [96.7 to 820.5 µmol TE/ 100g DSW (90% ethanol)] in selected seventeen seaweeds. There is significant difference ($p<0.05$) has shown in antioxidant properties obtained from FRAP and ORAC assay which shows that all seaweeds are significantly different in terms of antioxidant activities. The antioxidant activities are present mostly due to polyphenols as already viewed in literature review in Chapter 1. From the analysis, the seaweeds which show low to moderate antioxidant activities (0.09 to 6.0 mmol FE (FeSO4 eq.) /100g DSW) (Mili-Q water), (0.1 to 20.0 mmol FE /100g DSW) (90% ethanol) gave high TP content. Those species are *Callophyllis lambertii, Codium galeatum, Curdica angustata, Cystophora platylobium, Durvillaea potatorum, Macrocystis angustifolia, Macrocystis pyrifera, Melanthalia obustata, Phacelocarpus sp, Phyllospora comosa, Sargassum axillaris, Sierococcus*

axillaris and *Carpoglossum confluens.* Some of the seaweed species showed high antioxidant activities e.g. *Ecklonia radiata* (28.3 mmol FE /100g DSW) (Mili-Q water), Ecklonia *radiata* (10g) (29.7 mmol FE / 100g DSW) (Mili-Q water) but when *E.radiata* tested with 90% ethanol it nearly doubles the antioxidant activity of *E.radiata* (57.1 mmol FE/ 100g DSW) and for *E.radiata* (10g) gave (48.3 mmol FE /100g DSW) antioxidant activity. After that, *Cystophora monilifera* gave high antioxidant activity (13.5 mmol FE/ 100g DSW) with Mili-Q water but with 90% ethanol it showed (18.6 mmol FE /100g DSW). It was observed in general that some of the seaweeds have high phenolics content and high antioxidant activity in FRAP assay (Figure 3.3).

Although, most seaweeds gave low to moderate antioxidant activity but high phenolics content observed in most of seaweed species those tested with Mili-Q water e.g. *C.galeatum* (1968.1 mmol FE /100g TPC), *Phacelocarpus sp.* (2348.1mmol FE /100g TPC) and *Sierococcus axillaris* (2238.1 mmol FE /100g TPC). On the other hand, those seaweed species show high antioxidant activity those gave moderate total phenolics content e.g. *E.radiata* (521.1mmol FE/100g TPC) with mili-Q water and (1403.1 mmol FE /100g TPC) with 90% ethanol. According to Payne et al. (2013), FRAP assay measures only the reducing capability based on ferric ion which is not relevant to antioxidant activity mechanistically and physiologically.

The method has a disadvantage of some polyphenols reacting slowly and requiring longer reaction times for detection. FRAP assay is based on the hypothesis that redox proceeds so rapidly and all reactions complete within few minutes. In addition, FRAP assay cannot detect species that act by radical quenching (H transfer), particularly SH group-containing antioxidants. As a result, these seaweeds contain antioxidant activity with certain mechanisms that could not be detected by Ferric reducing antioxidant power (FRAP) assay. In the ORAC assay, antioxidant activities by oxygen radical absorbance capacity assay which ranged from 0.3 to 795 µmol TE/ 100g DSW (Mili-Q

water), 96.7 to 820.5 µmol TE/ 100g DSW (90% ethanol) for all seventeen seaweed species. The seaweeds containing low phenolics content gave moderate antioxidant activities which was comparable antioxidant activities with those which possess high phenolics content e.g. *Cystophora species, Sargassum species* and *Macrocystis species.* HAT method is dominant and it performed with electron transfer reaction and plays dominant role in logical redox reactions (Ou et al. 2002). ORAC assay a "total antioxidant activity assay", because it only measure antioxidant activity of peroxyl radicals. Moreover, low antioxidant activity was observed in some seaweed species with Mili-Q water which ranged 0.3 to 63.5 µmol TE/ 100g DSW but antioxidant value become moderate with 90% ethanol 80.5 to 545.5 µmol TE/ 100g DSW for the same seaweed species.

These seaweed species include *C.lambertii, C.angustata, C.confluens, C.angustata, C.platylobium, D.potatorum, M.angustifolia, M.pyrifera, Phacelocarpus sp.* and *S.axillaris. Ecklonia radiata, Sargassum sp.* and *C.monilifera* showed high antioxidant activities with both Mili-Q water and 90% ethanol. Total phenolics content (7779.1 to 256746.2 µmol TE/ 100g TPC) is much higher with 90% ethanol for most of seaweeds whereas water tested seaweeds gave very low TP content (156.2 to 93301.9 µmol TE/ 100g TPC) and antioxidant activities. The seaweed species have great significant potential from antioxidant activities. The previous research on some seaweeds by Andrew Lorbeer in 2014 showed antioxidant activities (FRAP 182 mmol FE /g DSW, ORAC 96.3 µmol TE /g DSW for *E. radiata,* FRAP 141 mmol FE /g DSW, ORAC 58.7 µmol TE /g DSW for *D. potatorum,* and FRAP 170 mmol FE /g DSW, ORAC 70.0 µmol TE /g DSW for *M.pyrifera*) which were less than results obtained in this thesis with 90% ethanol for the same seaweeds.

4.4 Differences in TP content, FRAP values and ORAC values with Mili-Q water & 90% ethanol

As discussion above and according to results in Table 3.2,3,4 large differences has been observed in TP content, FRAP activities and ORAC antioxidant activities with 90% ethanol as compared to water. In general, 90% ethanol tested seaweeds gave high total phenolics content, FRAP values and ORAC activity comparatively Mili-Q water. The reason is seaweeds polyphenols extracted by 90% ethanol conventional extraction method and the freeze dried crude extract when dissolved in Mili-Q water only some of contents dissolved and gave residue after centrifugation during preparation of samples. On the other hand, freeze dried crude extract of polyphenols totally soluble in 90% ethanol and no residue left after centrifugation.

As a result, seaweeds crude extracts of polyphenols are more soluble in 90% ethanol than Mili-Q water and due to high solubility gave large TP content, FRAP values and ORAC antioxidant activities. In case of Mili-Q water, in seaweeds extracts due to less solubility some of polyphenolic contents left to become soluble and seaweeds show low activity than 90% ethanol. But some water tested seaweeds e.g. *Cystophora monilifera* show nearly same activities as obtained with 90% ethanol, it means those seaweeds soluble in solvent and water as well.

4.5 Total phenolics content and antioxidant activity of *Ecklonia radiata* (10g) at large scale

Ecklonia radiata (C. Agardh) J. Agardh is one of the most abundant brown seaweed species in Southern Australia, constituting the largest fraction of biomass productivity. Other sixteen seaweeds have not been studied in the past for antioxidant activities, but *Ecklonia radiata* is well studied by

Charoensiddhi in 2014. As per previous research by Charoensiddhi in 2014, *E.radiata* show high TP content (4.4g PGEs /100g DSW) and antioxidant activities FRAP (29.7 mmol FE/100g DSW) and ORAC (740.1µmol TE/g DSW) which are nearly same as per results obtained from E.radiata (0.5g) and E.radiata (10g) in this project. But high total phenolics content and antioxidant activities was found by 90% ethanol comparatively Mili-Q water. Due to high total phenolics content (5.3g PGEs /100g DSW with Mili-Q water), (5.5g PGEs/100g DSW with 90% ethanol) and high antioxidant activities (FRAP 28.3mmol FE/100g DSW with Mili-Q water), (57.1mmol FE/100g DSW with 90% ethanol), (ORAC 712.8µmol TE/100g DSW with MQ water), (756.8 8µmol TE/100g DSW with 90% ethanol) obtained from 0.5g of *E.radi*ata extract.

Therefore, *E.radiata* is used for large scale production by using 10g of seaweed for analysis which give approximately similar total phenolics content [(4.3g PGEs/100g DSW with Mili-Q water), (4.5g PGEs/100g DSW with 90% ethanol)] and antioxidant activities [(FRAP 29.7mmol FE/100g DSW with MQ water) (48.5 mmol FE/100g DSW with 90% ethanol)] and [(ORAC 794.1µmol TE/100g DSW with MQ water), (ORAC 820.1µmol TE/100g DSW with 90% ethanol)].

4.6 Poor correlation between FRAP assay and ORAC assay with 90% ethanol than Mili-Q water

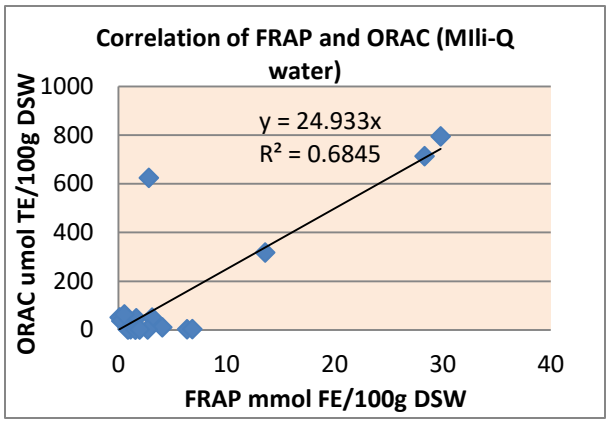
As mentioned in Figure 4.1(a, b) FRAP and ORAC assays are poorly correlated (R^2 = -0.259) after analysis with 90% ethanol and less correlated (R^2 = 0.6845) with Mili-Q water analysis. FRAP assay estimates the ferric reducing capacity while ORAC assay reflects the peroxyl radical scavenging activity. The weak correlation has been already discussed and explained well by giving the comparative study of total 927 freeze dried vegetable samples (Ou et al. 2002). The low relation of FRAP and ORAC assay was shown in antioxidant results from both assays for these species

Curdica angustata, Phyllospora comosa, Sargassum sp., Carpoglossum confluens which showed very low antioxidant activity in FRAP assay and high antioxidant activity in ORAC assay with 90% ethanol. In these assays the seaweed species belongs to same genera e.g. *Sargassum* species, *Cystophora* species and *Macrocystis* species give different antioxidant activities in both assays. Antioxidant activities are secondary metabolites in plants, which varied on stress conditions the plant encounter (Buricova & Reblova, 2008). Hence, significant differences were not found between antioxidant activities of the samples of same seaweed. However, two seaweed species growing in different environment may exhibit varied antioxidant activities because of the production of secondary metabolites different.

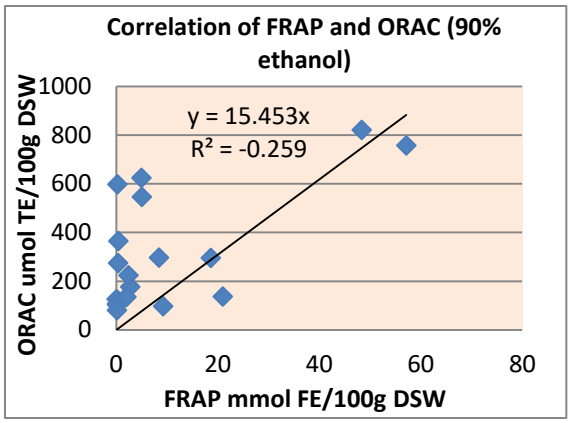
4.7 Good correlation between TP content with FRAP assay and ORAC assay with Mili-Q water than 90% ethanol

The correlation between total phenolics content and antioxidant activities in FRAP and ORAC values is presented in Figure 4.1 (c, d). There is positive linear correlation between FRAP value and total phenolics content for the seventeen Australian native seaweed species ($R^2 = 0.7056$) with Mili-Q water and ($R^2 = 0.5022$) with 90% ethanol. In the same way, in Figure 4.1(e, f) good correlation obtained between ORAC and total phenolics content with Mili-Q water ($R^2 = 0.881$) which is quite lower in case of 90% ethanol ($R^2 = 0.4816$). As a result, ferric reducing potential and oxygen reducing absorbance capacity can be related to phenolics content. From the data obtained, this research has identified several Australian native flowers which were rich sources of natural polyphenols and antioxidants for the potential applications of cosmeceuticals and health care products.

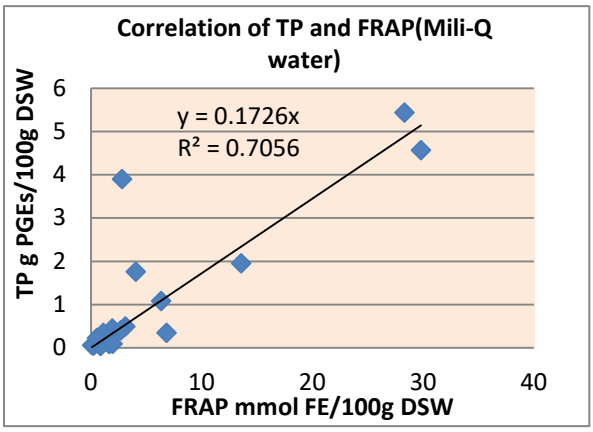
Figure 4.1: Correlation of FRAP and ORAC values (a, b) FRAP and TP content (c, d), ORAC and TP content (e, f) both with Mili-Q water and 90% ethanol of all seventeen selected Australian native seaweed species.



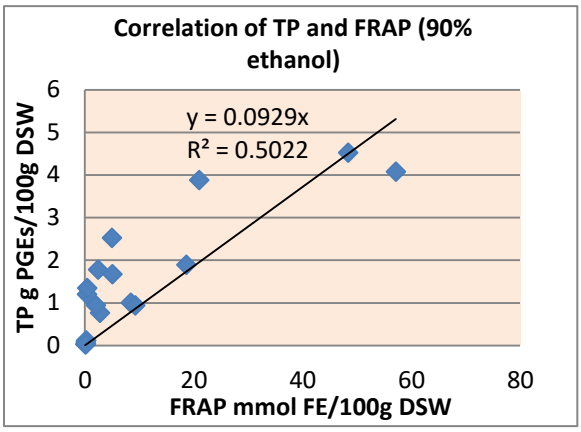
(a)



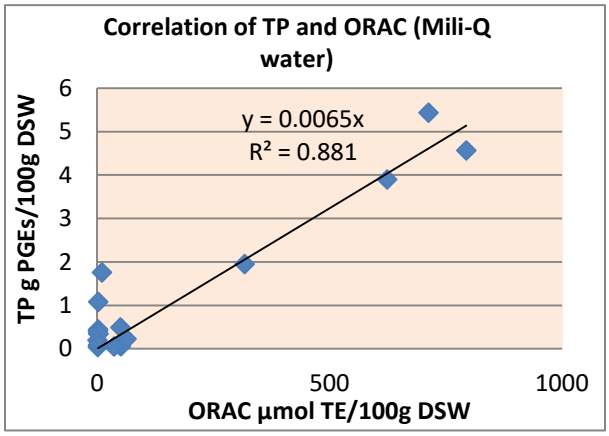
(b)



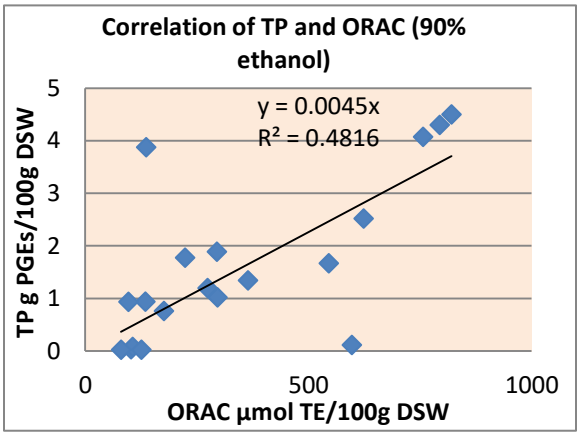
(c)



(d)



(e)



(f)

Chapter 5: Conclusion and Future directions

The work presented in this thesis illustrates the polyphenolic profiles of seventeen selected Australian native seaweed species. Two antioxidant assays FRAP and ORAC was used to determine the antioxidant activities from selected seaweeds. After 90% ethanol extraction the whole analysis of total phenolics content, FRAP assay, ORAC assay was done with Mili-Q water and 90% ethanol as well to get the accurate results. The conclusive results have been achieved to test the hypothesis of this project: the selected Australian native seaweeds possess excellent antioxidant activities with the potential to be used in cosmeceutical and health care industries.

Seventeen selected Australian native seaweeds were dried and extracted by 90% ethanol to obtain hydrophilic seaweed extracts followed by chemical characterization. Chemical based total phenolics content analysis conducted to determine polyphenols and FRAP assay and ORAC assay conducted to analyze the antioxidant activities from selected seventeen seaweeds. All these assays repeated with Mili-Q water and 90% ethanol to get the accurate results. Large variations were observed in the total phenolics content [0.3 to 5.4g PGEs /100g DW of seaweed with Mili-Q water] and [0.2 to 5.5g PGEs /100g DSW with 90% ethanol] in all seventeen seaweed species.

There were significant variations are observed in species which belongs to same genus. On the whole, four seaweeds *Ecklonia radiata*, *Cystophora monilifera*, *Sargassum sp*., and *Cystophora platylobium* gave high total phenolics conent and high antioxidant activities. There are great variations are seen in FRAP and ORAC assay, FRAP value varied from [0.09 to 29.5 mmol FE/100g DSW (Mili-Q water)], [1.4 to 57.5mmol FE/100g DSW (90% Ethanol)] and ORAC value varied from [0.3 to 795.1 µmol TE/ 100g DSW (Mili-Q water)], [96.7 to 820.5 µmol TE/ 100g DSW (90% ethanol)] in selected seventeen Australian native seaweeds. Antioxidant activities expressed in FRAP value/100g TP and ORAC value/100gTP of per unit total phenolics in the

seaweeds extracts exhibit different profiles from the antioxidant activity expressed in FRAP value/100g DSW and ORAC value/100g DSW. Poor correlation exists between the ORAC assay and FRAP assay illustrates that they determine the antioxidant activities in different mechanisms. Total phenolics content was found positively correlated with the FRAP assay and with ORAC assay. Total phenolics may contribute to the antioxidant capacity of FRAP assay and peroxyl scavenging activity of the ORAC assay.

In conclusion, this study successfully identified four South Australian native seaweed species which are best sources of antioxidant activities with the potential of commercial applications of healthcare and cosmeceutical products. Results data indicated that South Australian native seaweeds are potent natural resources in ferric reducing and radical scavenging capacities. These properties are beneficial for the development of nutritional products in pharmaceutical and in cosmeceutical industries.

Further studies are suggested to systematically evaluate their antioxidant efficacies in recent mechanisms and to characterize the bioactive compounds responsible for the antioxidant activities so they may be added in the industry formulation. Furthermore, the seaweed extracts with active ingredients in the antioxidant activities should be accounted for further toxicity tests on more skin models to find the safe dosage and possible side-effects to human beings. To conclude, this project has contributed a better understanding and new knowledge of the Australian native seaweeds in terms of their antioxidant activities with potential application in many different biotechnology industries.

6. References

AHN, B. R., MOON, H. E., KIM, H. R., JUNG, H. A. & CHOI, J. S. 2012. Neuroprotective effect of edible brown alga Eisenia bicyclis on amyloid beta peptide-induced toxicity in PC12 cells. *Archeology. Pharmaceutical. Research,* 35**,** 1989-1988.

AHN, G.-N., KIM, K.-N., CHA, S.-H., SONG, C.-B., LEE, J., HEO, M.-S., YEO, I.-K., LEE, N.-H., JEE, Y.-H., KIM, J.-S., HEU, M.-S. & JEON, Y.-J. 2006. Antioxidant activities of phlorotannins purified from Ecklonia cava on free radical scavenging using ESR and H2O2-mediated DNA damage. *European Food Research and Technology,* 226**,** 71-79.

AHN, G.-N., KIM, K.-N., CHA, S.-H., SONG, C.-B., LEE, J., HEO, M.-S., YEO, I.-K., LEE, N.-H., JEE, Y.-H., KIM, J.-S., HEU, M.-S. & JEON, Y.-J. 2007. Antioxidant activities of phlorotannins purified from Ecklonia cava on free radical scavenging using ESR and H2O2-mediated DNA damage. *European Food Research and Technology,* 226**,** 71-79.

AHN, G., LEE, W., KIM, K.-N., LEE, J.-H., HEO, S.-J., KANG, N., LEE, S.-H., AHN, C.-B. & JEON, Y.-J. 2015. A sulfated polysaccharide of Ecklonia cava inhibits the growth of colon cancer cells by inducing apoptosis. *EXCLI Journal,* 14**,** 294-306.

ALE, M. T., MARUYAMA, H., TAMAUCHI, H., MIKKELSEN, J. D. & MEYER, A. S. 2011a. Fucoidan from Sargassum sp. and Fucus vesiculosus reduces cell viability of lung carcinoma and melanoma cells in vitro and activates natural killer cells in mice in vivo. *International Journal of Biological Macromolecules,* 49**,** 331-336.

ALE, M. T., MIKKELSEN, J. D. & MEYER, A. S. 2011b. Important Determinants for Fucoidan Bioactivity: A Critical Review of Structure-Function Relations and Extraction Methods for Fucose-Containing Sulfated Polysaccharides from Brown Seaweeds. *Marine Drugs,* 9**,** 2106-2130.

ASKAROVA, S., YANG, X., SHENG, W., SUN, G. Y. & LEE, J. C. M. 2011. Role of Aβ-RAGE interaction in oxidative stress and cPLA(2) activation in astrocytes and cerebral endothelial cells. *Neuroscience,* 199**,** 375-385.

ATHUKORALA, Y., LEE, K.-W., SONG, C., AHN, C.-B., SHIN, T.-S., CHA, Y.-J., SHAHIDI, F. & JEON, Y.-J. 2003. POTENTIAL ANTIOXIDANT ACTIVITY OF MARINE RED ALGA GRATELOUPIA FILICINA EXTRACTS. *Journal of Food Lipids,* 10**,** 251-265.

BALBOA, E. M., CONDE, E., MOURE, A., FALQUÉ, E. & DOMÍNGUEZ, H. 2013. In vitro antioxidant properties of crude extracts and compounds from brown algae. *Food Chemistry,* 138**,** 1764-1785.

BARBOSA, M., VALENTÃO, P. & ANDRADE, P. 2014a. Bioactive Compounds from Macroalgae in the New Millennium: Implications for Neurodegenerative Diseases. *Marine Drugs,* 12**,** 4934.

BARBOSA, M., VALENTÃO, P. & ANDRADE, P. B. 2014b. Bioactive Compounds from Macroalgae in the New Millennium: Implications for Neurodegenerative Diseases. *Marine Drugs,* 12**,** 4934-4972.

BARROS, F. C. N., DA SILVA, D. C., SOMBRA, V. G., MACIEL, J. S., FEITOSA, J. P. A., FREITAS, A. L. P. & DE PAULA, R. C. M. 2013. Structural characterization of polysaccharide obtained from red seaweed Gracilaria caudata (J Agardh). *Carbohydrate Polymers,* 92**,** 598-603.

BENZIE IFF, STRAIN JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239:70–76.

BIXLER, H. J. & PORSE, H. 2010. A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology,* 23**,** 321-335.

BOURNE, K. Z., BOURNE, N., REISING, S. F. & STANBERRY, L. R. 1999. Plant products as topical microbicide candidates: assessment of in vitro and in vivo activity against herpes simplex virus type 2. *Antiviral Research,* 42**,** 219-226.

BURICOVA L., R. Z. 2008. Czech medicinal plants as possible sources of antioxidants. *Czech Journal of Food Science,* 26**,** 132–138.

BUTLER, A., SANDY, M. 2009. Mechanistic considerations of halogenating enzymes. *Nature,* 460**,** 848-854.

CABRITA, M. T., VALE, C. & RAUTER, A. P. 2010. Halogenated Compounds from Marine Algae. *Marine Drugs,* 8**,** 2301-2317.

CABRITA, M. T., VALE, C., RAUTER, A.P. 2010. Halogenated compounds from marine algae. *Marine Drugs* 8**,** 2301-2317.

CARDOZO, K. H., GUARATINI, T., BARROS, M.P., FALCAO, V.R., TONON, A.P., LOPES, N.P., CAMPOS, S., TORRES, M.A., SOUZA, A.O., COLEPICOLO, P., PINTO, E. 2007. Metabolites from algae with economical impact. *Comp Biochem Physiol C Toxicol Pharmacol,* 146**,** 60-78.

CHANDINI, S. K., GANESAN, P. & BHASKAR, N. 2008. In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chemistry,* 107**,** 707-713.

CHAROENSIDDHI, S., FRANCO, C., SU, P. & ZHANG, W. 2015. Improved antioxidant activities of brown seaweed Ecklonia radiata extracts prepared by microwave-assisted enzymatic extraction. *Journal of Applied Phycology,* 27**,** 2049-2058.

CHARLIER, R. H. & CHAINEUX, M.-C. P. 2009. The Healing Sea: A Sustainable Coastal Ocean Resource: Thalassotherapy. *Journal of Coastal Research***,** 838-856.

DE NYS, R., STEINBERG, P., WILLEMSEN, P., DWORJANYN, S., GABELISH, C., KING, R. 1995. Broad spectrum effects of secondary metabolites from the red alga Delisea pulchra in antifouling assays. *Biofouling* 8**,** 259-271.

DEVI, G. K., MANIVANNAN, K., THIRUMARAN, G., RAJATHI, F. A. A. & ANANTHARAMAN, P. 2011. In vitro antioxidant activities of selected seaweeds from Southeast coast of India. *Asian Pacific Journal of Tropical Medicine,* 4**,** 205-211.

DUREJA H, D. K., M GUPTA, V KUMAR, V LATHER 2005. Cosmeceuticals: An emerging concept. *Indian journal of Pharmacology,* 5**,** 155-159.

ELENA M. BALBOA, ENMA CONDE, M. LUISA SOTO, LORENA PÉREZ-ARMADA & DOMÍNGUEZ., H. 2015. Cosmetics from marine sources. *Application of maine biotechnology***,** 1015-1033.

EOM, S.-H., KIM, Y.-M. & KIM, S.-K. 2012. Antimicrobial effect of phlorotannins from marine brown algae. *Food and Chemical Toxicology,* 50**,** 3251-3255.

FARASAT, M., KHAVARI-NEJAD, R.-A., NABAVI, S. M. B. & NAMJOOYAN, F. 2014. Antioxidant Activity, Total Phenolics and Flavonoid Contents of some Edible Green Seaweeds from Northern Coasts of the Persian Gulf. *Iranian Journal of Pharmaceutical Research : IJPR,* 13**,** 163-170.

FISCHEL, J., LEMEE, R., FORMENTO, P., CALDANI, C., MOLL, J., PESANDO, D., MEINESZ, A., GRELIER, P., PIETRA, P., GUERRIERO, A 1995. Cell growth inhibitory effects of caulerpenyne, a sesquiterpenoid from the marine algae Caulerpa taxifolia. *Anticancer Research,* 15**,** 2155.

FLEURENCE, J. 1999. Seaweed proteins:: biochemical, nutritional aspects and potential uses. *Trends Food Science & Technology,* 10**,** 25-28.

GANESAN, P., KUMAR, C. S. & BHASKAR, N. 2008. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresource Technology,* 99**,** 2717-2723.

GLAZER, A. N. 1994. Phycobiliproteins—a family of valuable, widely used fluorophores. *Journal of Applied Phycology,* 6**,** 105-112.

GUINEA, M., FRANCO, V., ARAUJO-BAZÁN, L., RODRÍGUEZ-MARTÍN, I. & GONZÁLEZ, S. 2012. In vivo UVB-photoprotective activity of extracts from commercial marine macroalgae. *Food and Chemical Toxicology,* 50**,** 1109-1117.

HAGERMAN, A. E. 1988. Extraction of tannin from fresh and preserved leaves. *Journal of Chemical Ecology,* 14**,** 453-461.

HEO, S.-J., CHA, S.-H., KIM, K.-N., LEE, S.-H., AHN, G., KANG, D.-H., OH, C., CHOI, Y.-U., AFFAN, A. & KIM, D. 2012. Neuroprotective effect of phlorotannin isolated from Ishige okamurae against H2O2-induced oxidative stress in murine hippocampal neuronal cells, HT22. *Applied Biochemistry &. Biotechnology,* 166**,** 1520–1532.

HEO, S.-J., PARK, E.-J., LEE, K.-W. & JEON, Y.-J. 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresource Technology,* 96**,** 1613-1623.

HEO, S. J., KO, S.C., CHA, S.H., KANG, D.H., PARK, H.S., CHOI, Y.U., KIM, D., JUNG, W.K., JEON, Y.J. 2009. Effect of phlorotannins isolated from Ecklonia cava on melanogenesis and their protective effect against photo-oxidative stress induced by UV-B radiation. *Toxicol in vitro,* 23**,** 1123-1130.

HEO, S. J., LEE, K. W., SONG, C. B. & JEON, Y. J. 2003. Antioxidant activity of enzymatic extracts from brown seaweeds. . *Algae,* 18**,** 71-82.

HOLDT, K. 2011. Bioactive compounds in seaweed: functional food applications and legislation. *Journal of Applied Phycology,* 23**,** 543-597.

HONG YE, CHUNHONG ZHOU, YI SUN, XIN ZHANG, JUN LIU, QIUHUI HU & ZENG., X. 2009. Antioxidant activities in vitro of ethanol extract from brown seaweed Sargassum pallidum *European Food Research and Technology,* 230.

HUANG D, OU B, HAMPSCH-WOODILL M, FLANAGAN JA, PRIOR RL (2002) High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *Journal of Agriculture and Food Chemistry* 50:4437–4444

JAVANMARDI, J. & KUBOTA, C. 2006. Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. *Postharvest Biology and Technology,* 41**,** 151-155.

JIAO, G., YU, G., ZHANG, J. & EWART, H. S. 2011. Chemical Structures and Bioactivities of Sulfated Polysaccharides from Marine Algae. *Marine Drugs,* 9**,** 196-223.

JIMÉNEZ-ESCRIG A, GÓMEZ-ORDÓÑEZ E, TENORIO M, RUPÉREZ P (2013) Antioxidant and prebiotic effects of dietary fiber co-travelers from sugar Kombu in healthy rats. *Journal of Applied Phycology,* 25:503–512

JUNG, W.-K., HEO, S.-J., JEON, Y.-J., LEE, C.-M., PARK, Y.-M., BYUN, H.-G., CHOI, Y. H., PARK, S.-G. & CHOI, I. 2009. W. Inhibitory effects and molecular mechanism of dieckol isolated from marine brown alga on COX-2 and iNOS in microglial cells. *Journal of Agriculture and Food Chemistry,* 57**,** 4439–4446.

KANG, H. S., KIM, H. R., BYUN, D. S., SON, B. W., NAM, T. J. & CHOI, J. S. 2004. Tyrosinase inhibitors isolated from the edible brown algaEcklonia stolonifera. *Archives of Pharmacal Research,* 27**,** 1226-1232.

KANG, H. S., KIM, H.R., BYUN, D.S., SON, B.W., NAM, T.J., CHOI, J.S. 2004. Tyrosinase inhibitors isolated from the edible brown alga Ecklonia stolonifera. *Archrivesof Pharmacal Research* 27**,** 1226-1232.

KANG, K., LEE, K., PARK, J., LEE, N., NA, H., SURH, Y., YOU, H., CHUNG, M. & HYUN 2007. Triphlorethol-A induces heme oxygenase-1 via activation of ERK and NF-E2nrelated factor 2 transcription factor. *FEBS Lett,* 581**,** 2000-2008.

KANNAN, R. R. R., ADEROGBA, M. A., NDHLALA, A. R., STIRK, W. A. & VAN STADEN, J. 2013. Acetylcholinesterase inhibitory activity of phlorotannins isolated from the brown alga, Ecklonia maxima (Osbeck) Papenfuss. *Food Res. Int.,* 54**,** 1250–1254.

KAPRAUN, D. F., DUTCHER, J. A. & FRESHWATER, D. W. Quantification and characterization of nuclear genomes in commercial red seaweeds: Gracilariales and Gelidiales. *Hydrobiologia,* 260**,** 679-688.

KARINA H. M. CARDOZO, T. G., MARCELO P. BARROS, VANESSA R. FALCÃO, ANGELA P. TONON, NORBERTO P. LOPES, SARA CAMPOS, MOACIR A. TORRES, ANDERSON O. SOUZA, PIO COLEPICOLO. 2007. Metabolites from algae with economical impact. *Comparative biochemistry and physiology. Toxicology & pharmacology : CBP,* 146**,** 60-78.

KIM, A. R., SHIN, T.-S., LEE, M.-S., PARK, J.-Y., PARK, K.-E., YOON, N.-Y., KIM, J.-S., CHOI, J.-S., JANG, B.-C., BYUN, D.-S., PARK, N.-K. & KIM, H.-R. 2009a. Isolation and Identification of Phlorotannins from Ecklonia stolonifera with Antioxidant and Anti-inflammatory Properties. *Journal of Agricultural and Food Chemistry,* 57**,** 3483-3489.

KIM, S.-K. 2012. *Marine cosmeceuticals trends and prospects,* CRC press.

KIM, S.-K. 2014. Marine cosmeceuticals. *Journal of Cosmetic Dermatology,* 13**,** 56-67.

KIM, S.-K., THOMAS, N. V. & LI, X. 2011. Chapter 16 - Anticancer Compounds from Marine Macroalgae and Their Application as Medicinal Foods. *In:* SE-KWON, K. (ed.) *Advances in Food and Nutrition Research.* Academic Press.

KIM, W.-S., PARK, B.-S., PARK, S.-H., KIM, H.-K. & SUNG, J.-H. 2009b. Antiwrinkle effect of adipose-derived stem cell: Activation of dermal fibroblast by secretory factors. *Journal of Dermatological Science,* 53**,** 96-102.

KOIVIKKO R, LOPONEN J, HONKANEN T, JORMALAINEN V (2005) Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga Fucus vesiculosus, with implications on their ecological functions. J Chem Ecol 31:195–212

KUMAR, K. S., GANESAN, K. & RAO, P. V. S. 2008. Antioxidant potential of solvent extracts of Kappaphycus alvarezii (Doty) Doty – An edible seaweed. *Food Chemistry,* 107**,** 289-295.

KW, R. M. A. G. 1986. *In Progress in Phlorotannins, brown algal polyphenols,* Biopress Ltd: Bristol.

LEE, B. W. 2010. Cultivated seaweed and seaweed industry development in Australia. *Rural Industries Research and Development Corporation.*

LEE, S.-H. & JEON, Y.-J. 2013. Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. *Fitoterapia,* 86**,** 129-136.

LEE, S.-H., PARK, M.-H., HEO, S.-J., KANG, S.-M., KO, S.-C., HAN, J.-S. & JEON, Y.-J. 2010. Dieckol isolated from Ecklonia cava inhibits α-glucosidase and α-amylase in vitro and alleviates postprandial hyperglycemia in streptozotocin-induced diabetic mice. *Food and Chemical Toxicology,* 48**,** 2633-2637.

LI, Y.-X. & KIM, S.-K. 2011. Utilization of seaweed derived ingredients as potential antioxidants and functional ingredients in the food industry: An overview. *Food Science and Biotechnology,* 20**,** 1461-1466.

LIU, C., ZHANG, W., YANG, H., SUN, W., GONG, X., ZHAO, J., SUN, Y. & DIAO, G. 2014. A Water-Soluble Inclusion Complex of Pedunculoside with the Polymer β-Cyclodextrin: A Novel Anti-Inflammation Agent with Low Toxicity. *PLoS ONE,* 9**,** e101761.

LIU, H. & GU, L. 2012. Phlorotannins from Brown Algae (Fucus vesiculosus) Inhibited the Formation of Advanced Glycation Endproducts by Scavenging Reactive Carbonyls. *Journal of Agricultural and Food Chemistry,* 60**,** 1326-1334.

LJ., P. 1989. *Condensed tannins. In Natural Products of Woody Plants I,* Springer-Verlag: Berlin, Germany.

MCHUGH, D. 2003. A guide to the seaweed industry. *Food and Agriculture Organization of the United Nations.*

MEHRTENS, G. 1994. Haloperoxidase activities in Arctic macroalgae. *Polar Biology,* 14**,** 351-354.

MIYASHITA, K., HOSOKAWA 2008. *Marine nutraceuticals and functional foods,* CRC Press. Boca Raton, USA.

MORTON, S. A.-K. A. D. W. 2013. Cosmeceuticals Derived from Bioactive Substances Found in Marine algae. *Oceanography***,** 5-12.

MOHAMED S, HASHIM SN, RAHMAN HA (2012) Seaweeds: a sustainable functional food for complementary and alternative therapy. Trends Food Sci Tech 23:83–96

MYUNG, C.-S., SHIN, H.-C., BAO, H., YEO, S., LEE, B. & KANG, J. 2005. Improvement of memory by dieckol and phlorofucofuroeckol in ethanol-treated mice: Possible involvement of the inhibition of acetylcholinesterase. *Arch. Pharm. Res,* 28**,** 691-698.

NARASIMHAN, M. K., PAVITHRA, S. K., KRISHNAN, V. & CHANDRASEKARAN, M. 2013. In vitro Analysis of Antioxidant, Antimicrobial and Antiproliferative Activity of Enteromorpha antenna, Enteromorpha linza and Gracilaria corticata Extracts. *Jundishapur Journal of Natural Pharmaceutical Products,* 8**,** 151-159.

OU, B., HUANG, D., HAMPSCH-WOODILL, M., FLANAGAN, J. A. & DEEMER, E. K. 2002. Analysis of Antioxidant Activities of Common Vegetables Employing Oxygen Radical Absorbance Capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP) Assays: A Comparative Study. *Journal of Agricultural and Food Chemistry,* 50**,** 3122-3128.

PANGESTUTI, R. & KIM, S.-K. 2011. Neuroprotective Effects of Marine Algae. *Marine Drugs,* 9**,** 803-818.

PAYNE, A. C., MAZZER, A., CLARKSON, G. J. J. & TAYLOR, G. 2013. Antioxidant assays – consistent findings from FRAP and ORAC reveal a negative impact of organic cultivation on antioxidant potential in spinach but not watercress or rocket leaves. *Food Science & Nutrition,* 1**,** 439-444.

PEC, M. K., AGUIRRE, A., MOSER-THIER, K., FERNÁNDEZ, J.J., SOUTO, M.L., DORTA, J., DIÁZ-GONZÁLEZ, F.,

VILLAR, J. 2003. Induction of apoptosis in estrogen dependent and independent breast cancer cells by the marine terpenoid dehydrothyrsiferol. *Biochemistry Pharmacol,* 65**,** 1451-1461.

PANDEY, K. B. & RIZVI, S. I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity,* 2**,** 270-278.

PHILLIPS, J. A. 2001. Marine macroalgal biodiversity hotspots: why is there high species richness and endemism in southern Australian marine benthic flora? *Biodiversity & Conservation,* 10**,** 1555-1577.

S. KUMAR CHANDINI, P. GANESAN & BHASKAR., N. 2008. In vitro antioxidant activities of three selected brown seaweeds of India *Food Chemistry,* 107**,** 707-713.

SALAZAR, R., POZOS, M. E., CORDERO, P., PEREZ, J., SALINAS, M. C. & WAKSMAN, N. 2008. Determination of the Antioxidant Activity of Plants from Northeast Mexico. *Pharmaceutical Biology,* 46**,** 166-170.

SATHYA, R., KANAGA, N., SANKAR, P. & JEEVA, S. Antioxidant properties of phlorotannins from brown seaweed Cystoseira trinodis (Forsskål) C. Agardh. *Arabian Journal of Chemistry*.

SELBY, H. H., WHISTLER, R.L. 1993. *Industrial gums: Polysaccharides and their derivatives*.

SMIT, A. J. 2004. Medicinal and pharmaceutical uses of seaweed natural products: A review. *J Appl Phycol,* 16**,** 245-262.

STENGEL, D. B., CONNAN, S. & POPPER, Z. A. 2011. Algal chemodiversity and bioactivity: Sources of natural variability and implications for commercial application. *Biotechnology Advances,* 29**,** 483-501.

STERN, J. L., HAGERMAN, A. E., STEINBERG, P. D. & MASON, P. K. 1996. Phlorotannin-protein interactions. *Journal of Chemical Ecology,* 22**,** 1877-1899.

SULERIA, H. A. R., OSBORNE, S., MASCI, P. & GOBE, G. 2015. Marine-Based Nutraceuticals: An Innovative Trend in the Food and Supplement Industries. *Marine Drugs,* 13**,** 6336-6351.

TEIXEIRA, V., VALENTE, H., CASAL, S., PEREIRA, L., MARQUES, F. & MOREIRA, P. 2009. Antioxidant status, oxidative stress, and damage in elite kayakers after 1 year of training and competition in 2 seasons. *Applied Physiology, Nutrition, and Metabolism,* 34**,** 716-724.

THOMAS, N. V. & KIM, S.-K. 2011. Potential pharmacological applications of polyphenolic derivatives from marine brown algae. *Environmental Toxicology and Pharmacology,* 32**,** 325-335.

THOMAS, N. V. & KIM, S.-K. 2013. Beneficial Effects of Marine Algal Compounds in Cosmeceuticals. *Marine Drugs,* 11**,** 146-164.

URIKURA, I., SUGAWARA, T., HIRATA, T. 2011. Protective effect of fucoxanthin against UVB-induced skin photoaging in hairless mice. *Biosci Biotech Bioch,* 75**,** 757-760.

VAIRAPPAN, C. S. 2003. Potent antibacterial activity of halogenated metabolites from Malaysian red algae, Laurencia majuscula (Rhodomelaceae, Ceramiales). *Biomol Eng, ,* 20**,** 255-259.

WANG, T., JÓNSDÓTTIR, R., LIU, H., GU, L., KRISTINSSON, H. G., RAGHAVAN, S. & ÓLAFSDÓTTIR, G. 2012. Antioxidant Capacities of Phlorotannins Extracted from the Brown Algae Fucus vesiculosus. *Journal of Agricultural and Food Chemistry,* 60**,** 5874-5883.

WANG T, JÓNSDÓTTIR R, ÓLAFSDÓTTIR G (2009) Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem 116:240–248.

WEVER, R., HEMRIKA, W. 2001. *Vanadium haloperoxidases*, Encyclopedia of Inorganic and Bioinorganic Chemistry.

WIJESEKARA, I., YOON, N. Y. & KIM, S.-K. 2010. Phlorotannins from Ecklonia cava (Phaeophyceae): Biological activities and potential health benefits. *BioFactors,* 36**,** 408-414.

WIJESINGHE, W. A., J. P., J. & YOU-JIN 2011. Biological activities and potential cosmeceutical applications of bioactive components from brown seaweeds: a review. *Phytochemistry Reviews,* 10**,** 431-443.

WIJESINGHE, W. A. J. P. & JEON, Y.-J. 2012. Exploiting biological activities of brown seaweed Ecklonia cava for potential industrial applications: a review. *International Journal of Food Sciences and Nutrition,* 63**,** 225-235.

WIJESINGHE, W. A. J. P., JEON, Y.J. 2011. Biological activities and potential cosmeceutical applications of bioactive components from brown seaweeds: a review. *Phytochem Review,* 10**,** 431-443.

WINBERG, P., GHOSH, D., TAPSELL, L. 2009. Seaweed culture in integrated multi-trophic aquaculture: nutritional benefits and systems for Australia. *Rural Industries Research and Development Corporation*.

WOLFE, K. L. & LIU, R. H. 2007. Cellular Antioxidant Activity (CAA) Assay for Assessing Antioxidants, Foods, and Dietary Supplements. *Journal of Agricultural and Food Chemistry,* 55**,** 8896-8907.

WOMERSLEY, H. 1990. *Biogeography of Australasian marine macroalgae. in: Biology of Marine Plants,* Longman Cheshire ,Melbourne.

YAN, X., NAGATA, T. & FAN, X. 1998. Antioxidative activities in some common seaweeds. *Plant Foods for Human Nutrition,* 52**,** 253-262.

YE, H., ZHOU, C., SUN, Y., ZHANG, X., LIU, J., HU, Q. & ZENG, X. 2009. Antioxidant activities in vitro of ethanol extract from brown seaweed *Sargassum pallidum*. *European Food Research and Technology,* 230**,** 101-109.

YOON, N. Y., CHUNG, H. Y., KIM, H. R. & CHOI, J. S. 2008. Acetyl- and butyrylcholinesterase inhibitory activities of sterols and phlorotannins from *Ecklonia stolonifera*. *Fisheries Science,* 74**,** 200-207.

YUAN, Y. V. & WALSH, N. A. 2006. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. *Food and Chemical Toxicology,* 44**,** 1144-1150.

ZHAO, L., ZHAO, G., CHEN, F., WANG, Z., WU, J., HU, X. 2006. Different effects of microwave and ultrasound on the stability of (all-E)-astaxanthin. *J Agric Food Chem,* 54**,** 8346-51.

ZUBIA, M., ROBLEDO, D. & FREILE-PELEGRIN, Y. 2007. Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. *Journal of Applied Phycology,* 19**,** 449-458.

7.1 Appendix 3.2 Total phenolic content yield with two different solvents

The total phenolics content yield obtained by Phlorotannin assay of selected seventeen South Australian native seaweeds by using two different solvents (Mili-Q water and 90% ethanol). The yield expressed in grams phloroglucinol equivalents/ 100g of dry seaweed weight (DSW) and grams phloroglucinol equivalents/100g of seaweed extract with Mili-Q water and 90% ethanol respectively.

Table3.2 Total phenolics content yield of selected seventeen South Australian native seaweeds with two different solvents

Seaweed Species	**Mili-Q water**	**Mili-Q water**	**90% Ethanol**	**90% Ethanol**
	TP content (g PGEs /100g DSW)	**TP content (g PGEs /100g of seaweed extract**	**TP content (g PGEs /100g DSW)**	**TP content (g PGEs /100g of seaweed extract)**
Callophyllis lambertii [R]	0.05	1.15	0.03	0.67
Melanthalia obustata [R]	0.04	1.21	0.07	1.85
Phacelocarpus sp. [R]	0.03	0.48	0.02	0.3
Curdica angustata [R]	0.48	2.28	1.66	7.8
Codium galeatum [G]	0.08	0.88	0.02	0.25
Carpoglossum confluens	1.07	12.2	1.01	11.5
Cystophora monilifera	1.94	40.0	1.88	38.5
Cystophora moniliformis	0.34	5.51	0.93	15.0
Cystophora platylobium	1.75	10.5	3.87	23.3
Durvillaea Potatorum	0.45	9.58	0.76	16.5
Ecklonia Radiate	5.43	39.9	5.58	41.0
Macrocystis angustifolia	0.39	2.86	0.11	0.8
Macrocystis Pyrifera	0.34	6.81	1.19	23.9
Phyllospora Comosa	0.19	1.75	1.34	12.2
Sargassum sp.	2.92	26.5	3.35	30.5
Sargassum Axillaris	0.22	4.41	0.93	18.6
Sierococcus Axillaris	0.08	1.11	1.77	22.7

7.2 Appendix 3.3.1 Antioxidant activities by FRAP assay with two different solvents

Antioxidant activities obtained by FRAP assay of selected seventeen South Australian native seaweeds with Mili-Q water and 90% ethanol. The antioxidant activity expressed in mmol FeSO4 equivalents/100g of dry seaweed weight (DSW), mmol FeSO4 equivalents/100g of seaweed extract and mmol FeSO4 equivalents/100g of TPC with two solvents Mili-Q water and 90% ethanol respectively.

Table 3.3: Ferric reducing antioxidant activities of selected seventeen South Australian native seaweeds with two different solvents (means±SD, n=3)

Seaweed species	**Mili-Q water**	**90% Ethanol**	**Mili-Q water**	**90% Erhanol**	**Mili-Q water**	**90% Ethanol**
	FRAP VALUE(mmol FeSO4 eq./100g DW of seaweed)	**FRAP VALUE(mmol FeSO4 eq./100g DW of seaweed)**	**FRAP VALUE (mmol FeSO4 eq. /100g TPC)**	**FRAP VALUE (mmol FeSO4 eq. /100g TPC)**	**FRAP VALUE(mmol FeSO4 eq. /100g of seaweed extract)**	**FRAP VALUE(mmol FeSO4 eq. /100g of seaweed extract)**
Callophyllis lambertii [R]	0.09±0.0	0.17±0.0	180.15±0.0	556.8±0.0	2.07±0.0	3.75±0.0
Curdica angustata [R]	3.18±0.0	5.04±0.0	635.1±0.0	302.7±0.0	14.50±0.0	23.61±0.0
Melanthalia obustata [R]	0.24±0.1	0.14±0.0	498.4±0.1	191.6±0.0	6.06±0.1	3.54±0.0
Phacelocarpus sp. [R]	0.86±0.0	0.14±0.0	2348±0.0	614.6±0.0	11.35±0.0	1.84±0.0
Codium galeatum [G]	1.63±0.0	0.06±0.0	1968.8±0.0	283.6±0.0	17.39±0.0	0.70±0.0
Carpoglossum confluens	6.33±0.1	8.42±0.1	588.2±0.1	845.4±0.1	71.95±0.1	95.74±0.1
Cystophora monilifera	13.55±0.1	18.63±0.1	696.3±0.1	986.8±0.1	279.0±0.1	383.4±0.1
Cystophora moniliformis	6.82±0.0	9.24±0.1	1993.9±0.0	989.3±0.1	110±0.0	149.1±0.1
Cystophora platylobium	4.04±0.0	20.99±0.0	230.4±0.0	541.6±0.0	24.34±0.0	126.4±0.0
Durvillaea potatorum	1.92±0.0	2.76±0.0	436.6±0.0	363.3±0.0	41.8±0.0	60±0.0
Ecklonia radiata	28.3±0.1	57.1±0.1	521.1±0.1	1403±0.1	208.1±0.1	420.2±0.1
Macrocystis angustifolia	2.68±0.0	0.23±0.0	671.4±0.0	203.9±0.0	18.9±0.0	1.63±0.0
Macrocystis pyrifera	1.11±0.0	0.36±0.0	325.8±0.0	30.6±0.0	22.2±0.0	7.3±0.0
Phyllospora comosa	1.55±0.0	0.41±0.0	807.4±0.0	30.9±0.0	14.1±0.0	3.78±0.0
Sargassum sp.	2.79±0.1	4.96±0.1	71.84±0.1	196.9±0.1	25.4±0.0	45.1±0.0
Sargassum axillaris	0.53±0.0	2.0±0.1	240.8±0.0	214.6±0.1	10.6±0.0	39.9±0.1
Sierococcus axillaris	1.95±0.0	2.4±0.0	2238±0.0	136.1±0.0	25±0.0	30.96±0.0

7.3 Appendix 3.3.2 Antioxidant activities by ORAC assay with two different solvents

Antioxidant activities obtained by ORAC assay with 90% ethanol and Mili-Q water of selected seventeen South Australian native seaweeds. The antioxidant activities are expressed in µmol TE/100g of dry seaweed weight (DSW), µmol TE/100g of seaweed extract and µmol TE/100g of TPC with two solvents 90% ethanol and Mili-Q water respectively.

Table 3.4: Oxygen radical absorbance capacity (ORAC) of selected seventeen South Australian native seaweeds with two different solvents (means ± SD, n=3)

Seaweed Species	**Mili-Q Water**	**90% Ethanol**	**Mili-Q Water**	**90% Ethanol**	**Mili-Q Water**	**90% Ethanol**
	ORAC value (µmol TE/100g DSW)	**ORAC value (µmol TE/100g DSW)**	**ORAC value(µmol TE/100g of seaweed extract)**	**ORAC value (µmol TE/100g of seaweed extract)**	**ORAC value (µmol TE/100g of TPC)**	**ORAC value (µmol TE/100g of TPC)**
Callophyllis lambertii [R]	50.8±0.0	102.5±0.0	233.1±0.0	2164±0.0	93301.9±0.0	188173.9±0.0
Curdica angustata [R]	49.8±0.0	545.6±0.1	881±0.0	2552±0.1	10210.5±0.0	111766.4±0.1
Melanthalia obustata [R]	36.12±0.1	106.1±0.0	42.1±0.1	2590±0.0	72410.9±0.1	212876.7±0.0
Phacelocarpus sp. [R]	1.03±0.0	80.3±0.0	40.7±0.0	1057±0.0	2814.0±0.0	218689.6±0.0
Codium galeatum (G)	47.2±0.0	125.7±0.0	1250.8±0.0	1338±0.0	56881.9±0.0	151471.6±0.0
Carpoglossum confluens	2.0±0.0	296.2±0.0	1072.9±0.0	3367±0.0	194.0±0.0	27523.1±0.0
Cystophora monilifera	317.1±0.2	294.9±0.1	6525.7±0.2	6068±0.1	16287.3±0.2	15144.7±0.1
Cystophora moniliformis	2.6±0.0	96.7±0.0	13.6±0.0	1560±0.0	764.6±0.0	28277.9±0.0
Cystophora platylobium	10.0±0.0	136.4±0.0	502.4±0.0	822±0.0	574.4±0.0	7779.1±0.0
Durvillaea potatorum	1.96±0.0	176.2±0.0	5670.8±0.0	3832±0.0	444.0±0.0	39986.0±0.0
Ecklonia radiata	712.8±1.0	756.8±2.0	5241.2±1.0	5565±2.0	13125.0±1.0	13935.7±2.0
Macrocystis angustifolia	1.08±0.0	597.2±0.1	23.7±0.0	4206±0.1	269.4±0.0	149325.4±0.1
Macrocystis pyrifera	2.04±0.0	274±0.0	20.2±0.0	5480±0.0	597.3±0.0	80391.1±0.0
Phyllospora comosa	0.3±0.0	364.5±0.1	42.5±0.0	3314±0.1	153.6±0.0	189371.4±0.1
Sargassum sp.	623.7±2.0	623.7±1.0	60.7±2.0	5670±1.0	16019.2±2.0	16016.9±1.0
Sargassum axillaris	63.0±0.1	134.7±0.2	2.69±0.1	2673±0.2	28322.1±0.1	60520.7±0.2
Sierococcus axillaris	1.58±0.0	223.6±0.3	5473±0.0	2867±0.3	1809.9±0.0	256746.2±0.3