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Marine Bioactives from Saccharina latissima

Orlaith O'Connell
Department of Biological Sciences, Cork Institute of Technology

Marine Bioactives from *Saccharina latissima*

Orlaith O'Connell

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Head of Biological Sciences:

Dr. Brendan O'Connell

Academic Supervisors:

Dr. Helen O'Shea

Prof. Hugh McGlynn

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Declaration

I hereby declare that this thesis presented for the degree of Master of Science has not been previously presented for a higher degree to this or any other Institute. This thesis is of my own composition and any assistance provided is acknowledged in the text by reference to the researchers or their publications.

Signature: _____

ORLAITH O'CONNELL

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Abbreviations

- IBD – Inflammatory Bowel Disease
- GI – Gastrointestinal tract
- ROS – Reactive Oxygen Species
- PUFAs – Polyunsaturated Fatty Acids
- EAA – Essential Amino Acids
- FAO – Food & Agriculture Organisation
- WHO – World Health Organisation
- EPA - Eicosapentaenoic acid
- DHA - Docosahexaenoic acid
- SCFA – Short Chain Fatty Acids
- GIP - Glucose-dependent Insulinotrophic Polypeptide
- GLP-1 - Glucagon-like Peptide-1
- IL-8 – Interleukin 8
- TNF-α – Tumour Necrosis Factor
- IL-1β – Interleukin 1β
- IL-10 – Interleukin 10
- IL-6 – Interleukin 6
- MCP-1 – Monocyte Chemoattractant Protein 1
- IFN-y – Interferon Gamma y

Glossary

- Laver – an edible seaweed with thin sheet like fronds of a reddish purple/green colour which becomes black when dry
- Incretin – a metabolic hormone which stimulate a decrease in blood glucose levels
- Epiphytic – an organism which grows on the surface of a plant and derives its moisture & nutrients from the air, rain or water (in the case of marine environments)
- Chemokine – family of small cytokines secreted by cells with the ability to induce chemotaxis in nearby responsive cells
- Chemotaxis – the movement of an organism in response to a chemical stimulus

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Dedication

To

My Nana Betty & My Uncle Tom,

‘’Just a prayer from

The family who loved you,

Just a memory fond and true,

In our hearts

You will live forever,

Because we thought

The world of you.’’

Chapter 1

Marine bioactive from brown macroalgae and the potential role in gastrointestinal health

1. Introduction

Seaweeds can be classified into Rhodophyta (red), Chlorophyta (green) and Phaeophyta (brown) according to differences in pigmentation, structural and biochemical properties. These marine plants have typically been used by the food and cosmetic industries as sources of thickeners, gelling agents and stabilisers such as alginate, agar and carrageenan. However, current research has identified the vast potential of seaweed and seaweed extracts in promoting health. Not only are seaweeds concentrated sources of all essential nutrients, such as dietary fibres, proteins, vitamins, minerals and polyunsaturated fatty acids but a variety of extracts isolated from different seaweeds species have been shown to have rich bioactive potential (Dawczynski *et al*., 2007; Černá, 2011). Brown macroalgae in particular has a selection of bioactive compounds which would not be found in red or green seaweed including fucoidan, a sulphated polysaccharide mainly found in the cell wall of brown macroalgae, phlorotannin, a tannin only found in brown macroalgae, and fucoxanthin, a pigment which gives brown macroalgae its colour. These compounds have been linked to a variety anti-tumour, anti-inflammatory, anti-oxidant, anti-coagulant and anti-lipidemic effects which could be utilised in different food and pharmaceutical applications (Brown *et al*., 2014; Gupta & Abu-Ghannam, 2011; Wijesekara *et al*., 2011; Wijesinghe & Jeon, 2012)

An area which may benefit from these bioactives is the maintenance of gastrointestinal health. The gastrointestinal tract (GI) functions as a barrier to the entry of pathogens and is responsible for the absorption of essential nutrient and the regulation of hormones and is an important contributor to overall health and well-being. Defects in the biological function of the GI tract can lead to the development of severe gastrointestinal disorders such as inflammatory bowel disease (IBD). IBD, which includes Crohn's disease and ulcerative colitis, is characterized by chronic inflammation in the gastrointestinal tract. It is widely associated with urbanized societies and with Western style diets, which is characterized by highly processed and refined foods with a high content of sugar, fat, salt and protein from red meat. Along with environmental factors, genetic predisposition to IBD and dysregulation of the gut microbiota has been linked to the pathogenesis of this disorder (Corridoni *et al*., 2014). The prevalence of IBD is increasing worldwide and with long term affliction of IBD associated with increased risk of colorectal cancer, a major economic burden is placed on the global healthcare system. Thus, alleviatory and preventative measures are required to lessen this burden. Currently, there are several methods to treat the symptoms of IBD which include various anti-inflammatory

drugs, immune system suppressors and, in some severe cases, surgery. However, many of these methods have a high cost and severe side effects which may be unpleasant for patients. For this reason, natural compounds from sources such as plants and seaweed have been examined for anti-inflammatory properties similar to current anti-inflammatory drugs, but without any unwanted side effects.

This review will discuss bioactive compounds isolated from seaweed, with emphasis on those isolated from brown macroalgae, and the potential role they could play in the maintenance of health, particularly gastrointestinal health.

2. Cultivation of Seaweed

2.1 Global cultivation of seaweed

With increasing global populations, the demand for sustainable sources of raw materials remains a high priority. Many conventional sources of raw materials are non-renewable and have become vastly overburdened. Even some renewable sources have been utilised far beyond their regenerative abilities. Therefore, replacing these over-exploited resources with sustainable alternatives is crucial. The world's oceans cover 71% of the Earth's surface and host a wide range of biodiverse niches which have remained largely untapped. Although the ocean remains a significant source of food, increasing numbers of fish stocks have become over-exploited and are even in danger of becoming extinct, which has led to an increase in fish farming and aquaculture operations. In 2015, world aquaculture production reached 106 million tonnes live weight with a total estimated value of 163 billion US dollars (FAO, 2015). This total production was composed of farmed aquatic animals, aquatic plants and non-food products (Table 1.1). Aquatic plant aquaculture has developed in recent years due to the search for innovative sources of raw materials for the food and pharmaceutical industry. For example seaweed has generated interest as novel sources of protein and healthy food supplements, as well as raw materials for other industrial applications.

The use of seaweed by humans has been in practice for a long time. The remains of seaweed found on a 14,000 year old site in southern Chile have suggested that inhabitants of the site used seaweed for both human consumption and medicine (Dillehay *et al*., 2015). Asia in particular has a long history of seaweed usage outside of human consumption. Some early examples of medicinal uses of seaweed in Asia include the use of brown seaweed for goitre and the use of *Saccharina* strips in difficult

births to dilate the cervix (The Seaweed Site, 2014). Currently, Asian countries such as China and Indonesia are the largest producers of seaweed with a combined production of 25 million tonnes in 2015 (Figure 1.1), (FAO, 2017). While seaweed production in Asia is well-established due to tradition and taste, the use of seaweed in Europe is not as recognized and has largely been limited to the extraction of hydrocolloids such as alginate, carrageenan and agar. However in recent years, the drive towards novel functional ingredients for food and pharmaceutical products has shifted industry focus from hydrocolloid extraction to the refinement of high value marine bioactives.

Seaweeds produce a diverse biomass which can be used in a variety of formats i.e. fresh, dried, extracts or salted for direct consumption or for further processing into food additives, animal feeds, fertilisers, cosmetic products or functional foods (Rajapakse & Kim, 2011; Škrovánková, 2011; Anis *et al*., 2017). As a result, global demand for seaweed has increased along with increased usage of seaweed beyond traditional applications. Unlike the lead seaweed producing countries with established seaweed farming practices, the European seaweed industries rely mainly on the harvesting of natural resources (Mac Monagail *et al*., 2017). This could become an issue for future sustainability as comprehensive information regarding the regenerative properties of seaweed beds is lacking in many relevant species. The European Marine Biotechnology (MBT) ERA-NET published a marine biotechnology research and innovation roadmap identifying biomass production and processing as one of five key areas in the further development of marine biotechnology (Hurst, 2013). Some long term challenges facing this area include the sustainable harvesting of marine bio-resources including macroalgae, as well as the development of in-land and marine aquaculture and the improved extraction of high-value compounds from marine biomass.

In a bid to promote the growth of marine biotechnology, several European projects have been funded to tackle several of these challenges. One such strategy is the European funded At-Sea project, which ended in 2015 and aimed to develop novel technical textiles in order to demonstrate the economic and technical feasibility of open sea cultivation of macroalgae (http://www.atsea-project.eu/). Due to the success of this project, a spin-off company ‘’At Sea Technologies’’, which sells and develops sustainable turnkey seaweed farms, was co-founded by eight of the partners involved in the original projects (ATSEA Technologies, 2018). The SWAFAX project, which ended in 2013, was financed in order to develop extraction techniques for the production of food grade bioactives from macroalgae species and to evaluate the bioavailability of

these bioactives using *in vitro* and short term human trials. The SWAFAX project produced several food grade polyphenol rich bioactives from the brown seaweed *Ascophyllum nodosum* and investigated the bioavailability of these bioactives in humans along with any additional health benefits (Corona *et al*., 2016).

Table 1.1: World aquaculture production in 2015

	Quantity (live weight)	Value (first sale)
Food fish*	76.6 million tonnes	US$157.9 billion
Aquatic plants**	29.4 million tonnes	US$4.8 billion
Non-food products	41.1 thousand tonnes	US$208.2 million
Total	**106 million tonnes**	**US$163 billion**

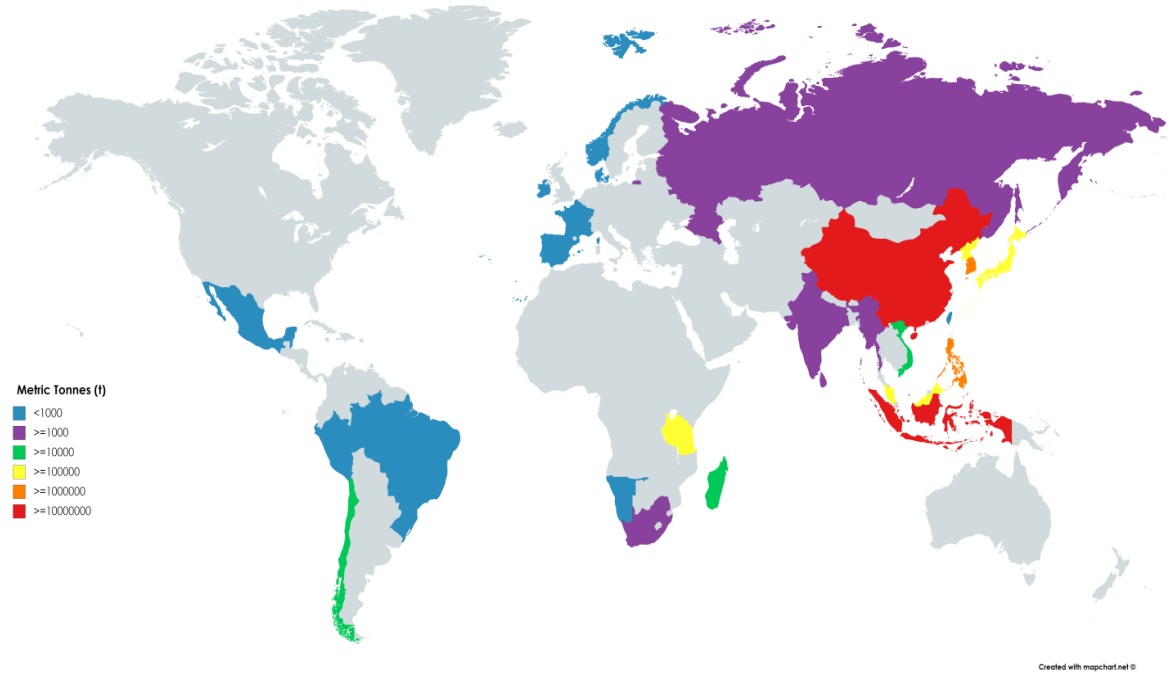


Figure 1.1: Seaweed production by aquaculture in 2015. Colour scale in metric tonnes (t). Source: FAO

* Food fish includes finfish, crustaceans, molluscs and other aquatic animals such as sea urchins and sea cucumbers, frogs and aquatic turtles, etc. Farmed crocodile and alligators are excluded.

** Aquatic plants include mostly seaweeds, plus some microalgae.

2.2 Cultivation of seaweed in Ireland

Many countries with large areas of coastline, including Ireland, could stand to benefit from increased seaweed cultivation. Predicted revenue from global marine biotechnology is expected to reach €1 billion by 2020, provided market growth of 6-8% per annum continues, and this is expected to create 10,000 new jobs (European Marine Board and Marine Biotechnology ERA- NET (2017)). At present, Ireland's seaweed and marine biotechnology sector is estimated to be worth €18 million annually (Morrissey *et al*., 2011). A report commissioned by Bord Iascaigh na Mara suggested that in order for the Irish seaweed sector to reach the expected value of €30 million per annum by 2020, the sector must capitalise on its wild resources as well as expand its seaweed aquaculture sites (Walsh & Watson, 2011). There are currently only a few licensed aquaculture sites in Ireland and much of the country's seaweed production is achieved through manual harvesting. The introduction of mechanised harvesting tools in order to intensify seaweed yield has generated interest due to a decline in young workers willing to engage in the hard and dirty work involved in seaweed harvesting. However, several wildlife conservation charities have raised concerns regarding the environmental impacts mechanical harvesting may have Ireland's native shores. Therefore harvesting trials should be conducted in order to investigate its impact on the regenerative capabilities of seaweed.

With increased interest in aquatic plants as functional ingredients, multiple strategies have been put in place to promote the development of sustainable aquaculture in Ireland. The Sea Change project, which took place from 2007-2013, aimed to develop Ireland's marine sector into a significant contributor to Irelands' economy. As part of this national project, industry scale hatcheries and growing trials were developed for four species of native seaweeds: *Palmaria palmata*, *Laminaria digitata*, *Saccharina latissima* and *Porphyra* sp. (Dring *et al*., 2013) (Figure 1.2). While *Palmaria palmata* and *Porphyra* sp. proved difficult to cultivate, through the modification of techniques used in Europe for related kelp species, *L. digitata* and *S. latissima* were successfully cultivated and grown out at sea, indicating the potential for future commercial cultivation of these seaweed species as a sustainable marine resource. Areas with invested interest in seaweed cultivation highlighted by this report included functional foods, cosmetics and pharmaceuticals.

According to the HARVEST Atlantic project many marine biotechnology companies surveyed in Ireland were concerned with fish, sea minerals and seaweed, with 38% of those companies involved in aquaculture and only 12% involved in research and development (Corcoran *et al*., 2014). Most of these marine biotechnology companies are actively involved in innovation in the form of new product development and marketing strategies, which has led to an increased range of products and improved quality of products. Currently, there are a range of Irish seaweed-based products available on the market which range from edible seaweeds, seasonings, snacks, teas, soaps, cosmetics, fertilisers and animal feeds. Many of these products can be categorised as high volume but low value products. Brown seaweeds, which are the most commonly cultivated seaweeds in Ireland, are comprised of a host of bioactive compounds which could be exploited by the food and pharmaceutical industry. Improved extraction and formulation of these compounds into value-added functional ingredients for functional foods, dietary supplements and pharmaceuticals will increase Ireland's share in the global marine biotechnology market, as a large quantity of Irish products from this sector are exported internationally to Europe and even further afield. Therefore adoption of the " biorefinery approach'', i.e. the successive extraction of valuable components from seaweed biomass, while leaving the remainder unmodified, in the seaweed industry has been suggested in order to achieve the maximum value from seaweed production (Balina *et al*., 2017).

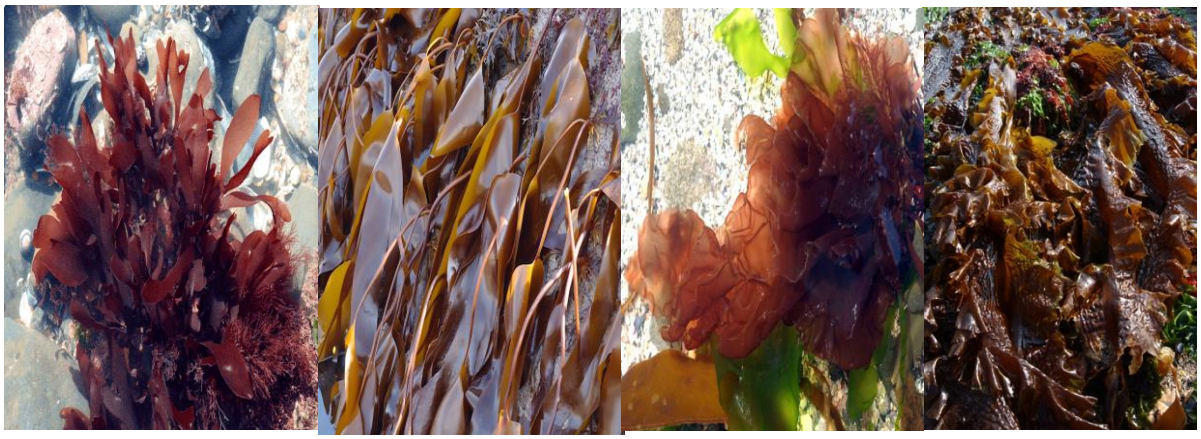


Figure 1.2: Illustration of some native seaweed species of Ireland. From left to right: *Palmaria palmata*, *Laminaria digitata*, *Porphyra spp* and *Saccharina latissima*

3. Seaweed as a preservative in the food industry

The global food market is continually growing and with rising exports to distant countries the demand for high quality, safe food is increasing. Preservatives are a fundamental component of meeting these demands. These compounds are used to maintain quality, extend shelf life and ensure the safety of fresh and processed foods through the inhibition of microbial growth and by preventing the release of reactive oxidative species (ROS). To ensure food safety and quality, a range of synthetic anti-microbial agents (weak organic acids, hydrogen peroxide and chelators) and synthetic anti-oxidant compounds (butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA), propyl gallate (PG) and tertbutyl hydroquinone (TBHQ) have been used as preservatives by the food industry (Brul & Coote, 1999; Shahidi, 2000). However, these compounds are suspected to be mutagenic and excessive intake has been found to cause liver damage. As a result, consumer preferences has started to shift from processed, ready to eat foods, towards additive free food or food with effective preservatives from natural sources (Tiwari *et al*., 2009). Terrestrial and marine plants are important sources for the extraction of natural preservatives which can be used alone or in conjunction with non-thermal preservation methods. Seaweed bioactives have been marked for use in nutraceutical and in functional foods, but these compounds have also been found to have potent anti-oxidant and anti-microbial properties, which indicates their potential as natural preservatives in the food industry

3.1 Seaweed as an antimicrobial agent

By nature many food products are perishable and subject to contamination by bacteria and fungi, which can cause undesirable reactions that affect the flavour, odour, textural and sensory properties of foods. Microbial contamination is a major concern as some microorganisms can cause foodborne illness. In 2014, for example, 5,251 food-borne outbreaks, including water-borne outbreaks, were reported in the EU, and bacterial toxins accounted for 16.1% of these outbreaks (European Food Safety Authority, 2015). In recent years, due to consumer concerns regarding synthetic additives in food and the rise of antibiotic resistance in some bacterial strains, numerous efforts have been made by the food industry to source natural compounds with potent antimicrobial properties.

In general, when compared to red and green seaweeds, brown macroalgae tends to have greater efficacy in inhibiting the growth of pathogenic bacteria. A study investigated the anti-bacterial activities of extracts from several native Irish seaweeds, namely,

Laminaria digitata, Laminaria saccharina, Himanthalia elongate, Palmaria palmata, Chondrus crispus and *Enteromorpha spirulina* against four common food spoilage bacteria *Listeria monocytogenes*, *Salmonella abony*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. While all seaweed extracts, excluding those from *C. crispus,* inhibited the growth of the bacteria it was determined that the brown seaweed species, *L. digitata, L. saccharina, H. elongata,* had significantly higher antimicrobial activities than the red and green species which has been linked to their total phenolic content (Cox, 2010).

Many seaweed extracts have shown potent antimicrobial activity against a number of Gram negative and Gram positive bacteria. The antibacterial activity of essential oils from four edible seaweeds - *Enteromorpha linza, Undaria pinnatifida, Laminaria japonica,* and *Porphyra tenera* against three strains of *L. monocytogenes,* a virulent bacterium which causes severe foodborne illness was investigated. It was determined that the essential oil isolated from the green macroalgae *E. linza* had a greater inhibitory action as it inhibited the growth of all three strains of Listeria rather than just the two strains seen in the other essential oils (Patra & Baek, 2016). Another example includes the extract polyhydroxylated fucophlorethol, from the brown macroalgae *Fucus vesiculosus,* which displayed antibacterial activity against both the Gram negative bacteria *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and the Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* (Sandsdalen *et al*., 2003). In contrast Gupta *et al* (2010), found that the methanol extracts of *Himanthalia elongata,* S*accharina latissima* and *Laminaria digitata* had a more pronounced anti-bacterial effect against Gram negative bacteria (*S. albany* and *P. aeroginosa*) when compared with Gram positive bacteria (*L. monocytogenes* and *E. faecalis*). It was also determined that the application of heat to these extracts caused degradation of their anti-microbial activities. As anti-microbial properties of these bioactives are dependent on the extraction method and seaweed species of origin, more research should be carried out to isolate an appropriate method to create effective anti-microbial extracts.

3.2 Seaweed as an anti-oxidant

Anti-oxidants have a long history of use in the food industry. Increased global urbanization has generated the need for high-quality products with extended shelf-lives and improved ability to survive extended transportation. One of the major challenges for the food industry to reach these goals is the prevention of oxidation. Lipid oxidation

negatively influences many food characteristics including flavour, nutritional quality, texture and colour. The presence of reactive oxygen species (ROS) such as superoxide anions and the hydroxyl radical are common causes of food decay and rancidity. Anti-oxidants such as BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisol) and TBHQ (tertbutyl hydroquinone) are used to prevent oxidation in foods with both high lipid contents, such as vegetable oils, animal fats and processed meats, and in foods with low lipid contents, such as cereal and grains. Currently there is interest in finding natural anti-oxidants which are effective enough to substitute commercial synthetic anti-oxidants due to consumer interest in minimally processed, additive free food products.

The use of seaweeds as a natural preservative is of interest due to their rich antioxidant capabilities. A strong correlation between phenolic content and antioxidant activity of seaweed extracts has been elucidated by several studies. A study of the antioxidant potential of eight Malaysian seaweed species found that extracts from two green seaweeds (*Caulerpa lentillifera* and *C. racemose*) and brown seaweed (*S. polycystum*) had a greater radical scavenging ability and antioxidant reducing power than other seaweed species assayed. These antioxidant capabilities were correlated with the high level of phenol content in each species (Matanjun *et al*., 2008). Additional metabolites in brown seaweed have been identified to have potent antioxidant activities. The marine carotenoid fucoxanthin and its metabolite fucoxanthinol were found to have potent radical scavenging activities, with scavenging activity of fucoxanthin 13.5 times higher than α-tocopherol, an anti-oxidant which is absorbed and accumulated in humans (Sachindra *et al*., 2007).

However, when investigating extracts from seaweed for antioxidant properties, the extraction method should be taken into consideration. Both processing and extraction methods have an effect on the total phenolic content and antioxidant ability of the seaweed species. A study investigated the effect of processing and extraction methods on antioxidant activities of extracts from the red macroalgae *Porphyra tenera.* In the study, the laver was dried, roasted or seasoned and extracted by means of hot water extraction or ethanol extraction. It was determined that antioxidant activity of dried, roasted and seasoned laver increased in a concentration - dependent manner while ethanol extracts had higher free radical scavenging abilities when compared to water extracts (Hwang & Thi, 2014). A similar study investigated the effect of hydrothermal processing on antioxidant and free radical capability of edible Irish brown seaweed, *Laminaria saccharina, Laminaria digitata and Himanthalia elongata*. When compared

with raw samples of the same seaweed species it was determined that both total phenolic content and free radical scavenging abilities were increased after hydrothermal processing (Rajauria *et al*., 2010).

4. Environmental effects on brown macroalgae growth & composition

Brown macroalgae, also known as Phaeophyta, is one of the largest and most complex classes of seaweed. Its characteristic brown colour is due to the presence of fucoxanthin, a pigment which is not found in any other class of seaweed. Like other macroalgae, brown macroalgae has a broad distribution, from tropical to temperate climates. Brown macroalgae species such as *Laminaria hyperborean* and *Ascophyllum nodosum* are often used in industry as sources of alginates, derivatives of alginic acids, which are commonly used as stabilizers, emulsifiers and binding agents. Recent research has identified several bioactive compounds in brown macroalgae which could be used in a host of applications in the food and pharmaceutical industry. However, a barrier to consistent functional ingredients is the variable composition of seaweeds throughout the year. Because of the growth environment, seaweeds are often exposed to varying degrees of environmental conditions. Seaweed composition, yield and biomass often varies according to harvesting season, temperature, salinity and light intensity which would prove a problem for consistent extraction of novel bioactives. Therefore in-depth seasonal studies are required to not only evaluate the effect of this variation on composition of seaweed extracts but to determine the best harvesting strategy for each species.

4.1 Seasonal change

Harvesting season is an important concept in the seaweed industry. During seaweed aquaculture, sporocytes are typically seeded in ropes or nets and then fixed at certain depths in the sea. Harvesters continually examine these ropes to determine optimum biomass for harvesting. Similar to terrestrial plants, seaweeds have seasons in which peak growth is achieved. This season can often differ, depending on seaweed species and method of growth. For example, *Sargassum polycystum*, a brown macroalgae common to Indian waters, was harvested from the wild and found to achieve maximum growth during the winter months (Srinivasa Rao & Umamaheswara Rao, 2002). In contrast, the cultivated kelp species *Saccharina latissima* demonstrated optimal growth in autumn and spring (Handå *et al*., 2013).

The variability of seaweed composition and extract bioactivity is also largely influenced by harvesting season. A study investigating the nutritional composition and anti-proliferative activity of *Sargassum oligocystum* samples from four different seasons in Thailand found that, in general, nutritional content was high during the hot dry and early monsoon season (February and May). Ethanolic and lipophilic extracts from the monsoon season (August) were shown to have a more effective anti-proliferative activity against a lung cancer cell line. Similar studies have found seasonal variations in composition in other species of seaweed (Praiboon *et al*., 2017). Marinho *et al*., (2015), found that total lipid content of *Saccharina latissima* increased in winter and decreased in summer, a pattern which has been observed for other brown macroalgae species as well as in some red and green macroalgae (Nelson *et al*., 2002). Seasonal variation presents an obstacle to consistent isolation of bioactives such as fucoidan, due to their varying concentrations in seaweed throughout the year. A study carried out on three species of brown macroalgae - *Saccharina japonica*, *Sargassum pallidum*, and *Stephanocystis crassipes* demonstrated maximum fucoidan content in different time periods for each species which has been linked to the development of reproductive organs (Skriptsova, 2016). Much of the variation in seaweed composition may be caused by changes in environmental temperature, light intensity, salinity and presence of essential nutrients as caused by the changing of the seasons.

4.2 Water Temperature

Another possible contributing factor to the variability of seaweed biomass is the environmental temperature. Due to genetic adaption over millions of years, each seaweed species has an optimum temperature range which usually correlates with local temperature conditions. For example, Antarctic seaweed would have a narrow temperature range due to little variation in local temperature, while temperate seaweed species have one of the broadest temperature ranges due to larger seasonal changes in temperature (Buchholz *et al*., 2012). Within this temperature range growth is at its peak, with growth rapidly declining above this range.

Seaweeds have the ability to acclimatise growth and photosynthesis in response to daily/seasonal changes in ambient temperature. This phenotypic acclimatisation usually allows maximum growth at a broader temperature range and can vary between species. A recent study carried out on five common seaweed species from Atlantic Canada, including *Ascophyllum nodosum, Fucus vesiculosus, Chondrus crispus, Laminaria digitata* and *Codium fragile* ssp. *Tomentosoides,* investigated the effect of an increase in

environmental temperature on the growth and survival of these species. It was determined that the kelp species, *L. digitata*, followed by the rockweed species, *Ascophyllum nodosum*, *F. vesiculosus*, had the worst growth and survival in higher temperatures, while the red and green seaweed species, *C. crispus* and *Codium fragile* ssp. *Tomentosoides* respectively, had high survival rates in all temperature conditions (Wilson *et al*., 2015). The low survival rate of kelp species beyond 20°C has been established in similar studies. A study carried out on the kelp species *S.latissima* collected from Norway, determined that this species can optimise photosynthesis at temperatures within the range of 10°C to 15°C (Andersen *et al*., 2013). However, beyond this range, poor performance and higher mortality rates were seen. Poor performance at high temperatures could be due to the degradation of essential proteins and enzymes in seaweeds.

Therefore, rising sea temperatures could present as a possible concern to the marine industry. A study carried out in Japan attempted to predict the continued effect of rising sea temperatures on the distribution of *Ecklonia cava,* a brown macroalgae which has been in decline in recent years due to global warming and heavy grazing by marine organisms (Takao *et al*., 2015). The study predicted the decline of *E .cava* populations at both high and low emission scenarios either through increased temperature stress or through increased grazing by marine herbivores. Rising sea temperatures may affect the natural distribution and performance of many seaweed species particularly brown macroalgae.

4.3 Light Intensity

Temperature effects on the growth of seaweeds are often linked with light intensity. Light is an essential component of any plant growth, due to its role in photosynthesis. Depending on the habitat of the seaweed species, growth could be dependent on high or low light requirements which can often correspond with environmental temperatures. Artic brown macroalgae species (i.e. *S. latissima*, *L. digitata*, *A. esculenta*) have adapted to grow and photosynthesize under very low temperatures and under low light intensities. However, exposure of the micro-stages of these Artic species to high levels of UV radiation along with high temperatures has been linked with reproduction and sporocyte formation (Müller *et al*., 2008).

Brown macroalgae have effective photo-protective responses to deal with high light stress. The xanthophyll-cycle, also known as the violaxanthin cycle, is an important

photo-protection mechanism found in most plants. During this cycle a reversible conversion reaction of the carotenoid violaxanthin by the intermediate antheraxanthin into zeaxanthin takes place. This allows photosynthetic light harvesting complexes to switch from light harvesting under low light conditions to light dissipating under high light conditions (Figure 1.3) (Jahns *et al*., 2009; Goss & Jakob, 2010). The efficiency of these protective mechanisms often depends on the habitat of the species. A study compared the light response of *Laminaria abyssalis*, which grows in deep waters and low light, and *L. digitata*, which grows in shallower waters and exposed to higher light intensities. It was determined that *L. abyssalis* had lower tolerance of high irradiation which may be due to its reduced xanthophyll-cycle pool size (Rodrigues *et al*., 2002). Brown macroalgae also produces several compounds of interest such as fucoxanthin and phlorotannins, which have been linked to protective responses to light induced oxidative stress (Cruces *et al*., 2013). However, the photo-protective activities of these compounds are often impaired by exposure to temperatures beyond normal growth range. Therefore, in a future scenario of higher sea temperatures and increased exposure to UV radiation, the growth and distribution of several key brown macroalgae species may be significantly altered.

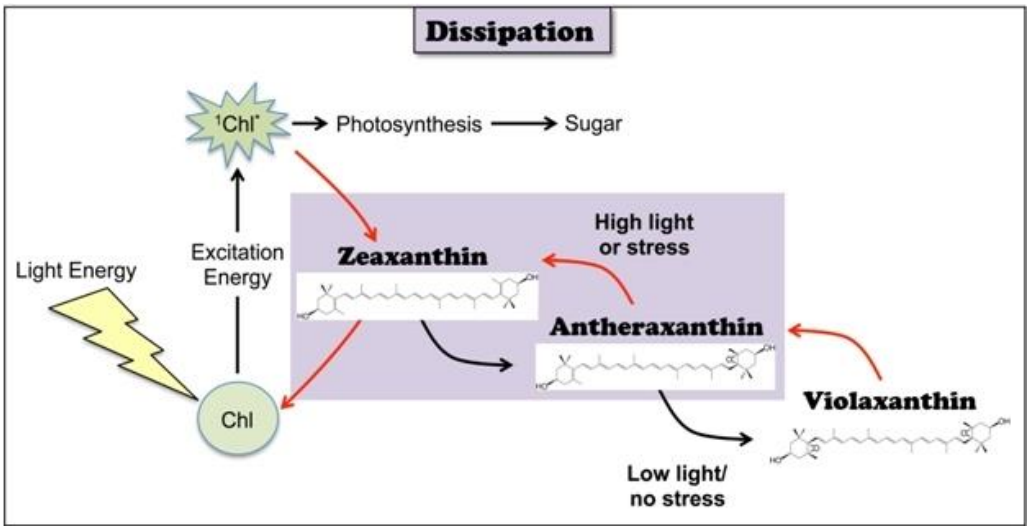


Figure 1.3: Photo-protection by dissipation of excess light energy aided by xanthophyll cycle carotenoids. The xanthophyll violaxanthin is converted to zeaxanthin (via the intermediate antheraxanthin) whenever chloroplasts absorb excess light. Zeaxanthin acts as a key facilitator of the dissipation of excess 1Chl*. Conversely, when light is not excessive, zeaxanthin is disengaged from energy dissipation and converted back to violaxanthin, thereby returning to an efficient utilization of light energy in photosynthesis (Nature.com, 2018)

4.4 Salinity

The growth, survival and distribution of marine macroalgae are often determined by their ability to tolerate environmental stresses. One such environmental stress is a change in ambient salinity. Salinity is a technical term used to describe the concentration of dissolved salts in a body of water. On average, the salinity of the open ocean is approximately 35 parts per thousand (ppt). Salinity gradually decreases as you move from tropic to polar seas due to lower levels of evaporation and increased freshwater sources. As with temperature and light intensity, salinity tolerance of marine macroalgae is often dependent on habitat. Many seaweed species grow in fixed positions, either attached to rocks or other hard substrata, and as such, they are exposed to fluctuations in salinity levels during low tides. As a result macroalgae have developed effective adaptive and tolerance strategies in response to variations in ambient salinity including extensive ROS detoxification, accumulation of solutes which maintains cellular membrane integrity and adjust cellular osmotic content (i.e. amino acids such as proline, carbohydrates such as sucrose and polyols and quaternary ammonium compounds such as glycine betaine and proline betaine) and altered ion homeostasis (Kumar *et al*., 2014).

Due to climate change, ambient salinity may change in some regions, leading to shifts in macroalgae distribution. In South American regions, increased precipitation has led to increased levels of freshwater, thereby causing a decline in salinity levels. Growth of *Sargassum stenophyllum,* brown seaweed common to this region, was found to be negatively affected by changes in salinity levels, indicating potential population shifts in the marine community (Scherner *et al*., 2013). Prolonged exposure to reduced ambient salinity has been linked to inhibition of photosynthesis and reduced growth (Connan & Stengel, 2011). Ambient salinity is also a contributing factor towards industrial utilization of marine bioactives as habitat often influences the yield and composition of seaweed. For example, fucose-containing sulphated polysaccharides from brown algae have been linked with distinct health-promoting properties; prompting industry focus on availability and quality of these bioactives. Ehrig & Alban., (2015) found that *S.latissima* harvested from the North Atlantic in autumn had a higher yield of fucose-containing sulphated polysaccharides than *S.latissima* harvested from the Baltic Sea which has a much lower salinity. In conclusion, when harvesting seaweed for the targeted isolation of specific bioactives, habitat as well as harvesting season should be considered as influencing factors on yield and bioactivity of the compounds

4.5 Biofouling

Like other eukaryotic organisms, marine macroalgae host a complex and diverse community of microorganisms with essential roles in health and defence (Singh & Reddy, 2014). Epiphytic bacterial communities in particular have essential roles in normal growth and morphology of algae species. A study carried out on the green macroalgae *Ulva fasciata* determined that when the macroalgae were cultured in an axenic environment, abnormal morphology developed (Singh *et al*, 2011). The addition of bacteria isolates from different *Ulva* species induced normal morphology, indicating a symbiotic relationship between the development of the macroalgae species and its bacterial community.

However, not all marine microorganisms are beneficial towards seaweed growth. Biofouling, which is a term used to describe the accumulation of microorganisms, plants or animals on wetted surfaces, is a major barrier which can prevent year round cultivation of macroalgae. Encrusting bryozoan species in particular are a challenge in the cultivation of kelp species such as *Saccharina latissima*. These microorganisms develop colonies on the surface of the kelp, weakening its structure and making its blades more prone to breakages. Prolonged exposure to the invasive bryozoan *Membranipora membranacea* decreases tissue strength in kelp species, thereby reducing the marine plants' ability to withstand waves (Krumhansl *et al*., 2011). A study determined that the settlement period of two encrusting bryozoan species *M.membranacea* and *Electra pilosa* on kelp occurred around mid-June, with rapid colonization observed in late June and July (Figure 1.4), (Førde *et al.,* 2016). In order to avoid seasonal biofouling and harvest the optimum seaweed biomass, seaweed producers are often restricted in their harvesting time.

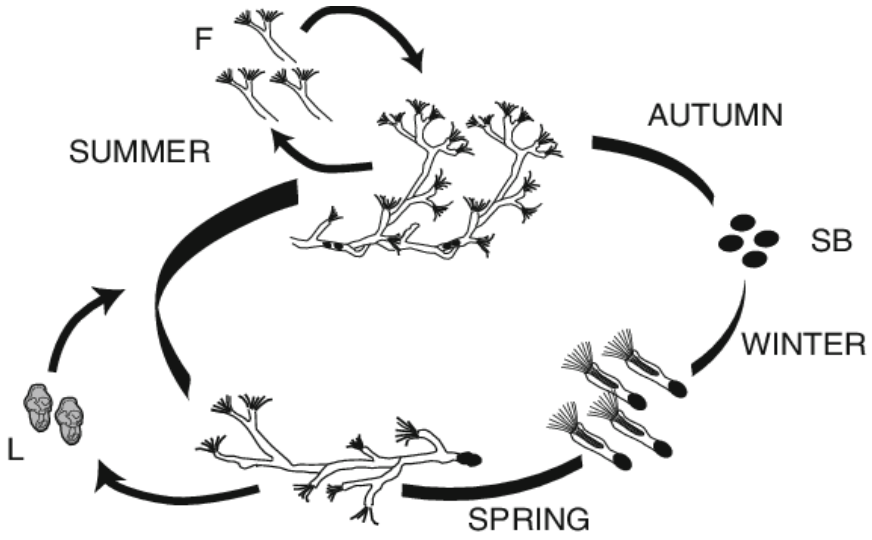


Figure 1.4: Seasonal lifecycle of bryozoan species. During winter months bryozoan survive in resting stages (statoblasts). Proliferation occurs in spring and summer months. During summer months bryozoan larvae are released and colonies grow rapidly and disperse by fragmentation and re-attachment of branches. SB=statoblasts, L= larvae, F=fragmentation. (Okamura et al, 2015)

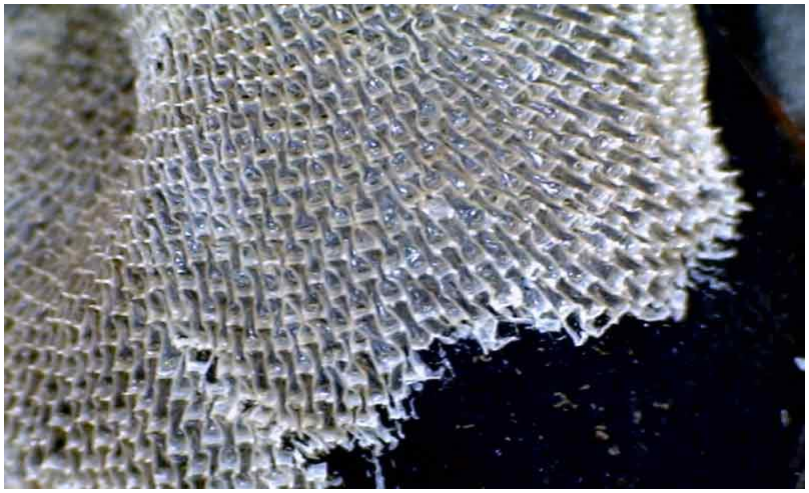


Figure 1.5: Close up view of *Membranipora membranacea* colony

5. Nutritional benefits of brown seaweed

Many countries in which seaweed is a staple in the diet have higher life expectancies and lower rates of morbidity and disease. The island of Okinawa has one of the highest life expectancy rankings in Japan, along with lower rates of age associated diseases. A strong correlation has been identified between these lower disease rates and the typical Okinawa diet which is low calorie, nutrient dense and reduced in meat, saturated fats, sugar and dairy products. A study carried out on Okinawan immigrants living in Brazil identified higher obesity rates and higher hypertension than their counterparts living on the island, which may be linked to changes in lifestyle and therefore dietary habits (Moriguch *et al*., 2004). More than a dozen varieties of seaweed, including *kombu*, *wakame* and *nori*, are commonly used in the Okinawa diet which may contribute towards these health effects. Nutritional analyses of different seaweed species have identified the marine algae as low calorie sources of all essential nutrients, many of which have been marked with bioactive potential for use in the food, feed and cosmetic industry.

5.1 Carbohydrates & Dietary Fibre

Seaweeds are a rich source of carbohydrates and dietary fibre. The typical polysaccharides found in brown seaweeds, which includes cellulose, laminarin, mannitol and alginate, cannot be digested by human digestive enzymes and therefore makes seaweed an important source of dietary fibre. The benefits of fibre consumption have been well documented in scientific literature, particularly in digestive health. Dietary fibre has been linked with the prevention of Type II diabetes, obesity, inflammation and certain types of cancers (Kaczmarczyk *et al.,* 2012). Approximate total dietary fibre content in edible seaweed has been estimated to be within the range of 36-62% dry weight, of which a large quantity consists of soluble fibre (Dawczynski *et al*., 2007; Gómez-Ordóñez *et al*., 2010).

Fibre can be sub-classified into soluble and insoluble fibres depending on their degree of solubility. Soluble fibres are easily fermentable fibres, which form a gel when dissolved in water, while insoluble fibres provide bulking action and tend to be only fermented by anaerobic bacteria in the colon. Consumption of soluble fibre has been shown to reduce cholesterol levels, lower blood pressure, improve digestive disorders and improve weight management through delayed gastric emptying (Anderson *et al*., 2009). The immunomodulatory effects of soluble fibres and resistant starches have also

been investigated in animal models of inflammation, demonstrating the potential of soluble fibre in IBD therapies (Bassaganya-Riera *et al*., 2011). Much of these health benefits can be attributed due to the role of soluble fibre in short chain fatty acids (SCFA) production. Both soluble and insoluble fibres are fermented by the microbiota in the gut to produce quantities of SCFA which have roles in the reduction of inflammation, improved barrier function of the gut and regulation of gut hormones (Peng *et al*., 2007; Tedelind *et al*., 2007; Psichas *et al*., 2015). As seaweed has high quantities of fibre, particularly soluble fibre, their potential for incorporation into functional products aimed at the improvement of health has generated increasing amounts of interest. For example *Ulva ohoni,* a green seaweed rich in soluble fibre and magnesium, has been found to reduce symptoms of metabolic syndrome in rat models more effectively when compared with *Derbesia tenuissima*, which had high levels of insoluble fibre (Kumar *et al*., 2015).

5.1.1 Seaweed polysaccharides as prebiotics

Seaweed carbohydrates have also been identified as emerging sources of prebiotics. The gastrointestinal tract hosts a complex microbial ecosystem which supports normal function of the gut. Imbalances in this microbial community has been observed in many chronic diseases related to the gastrointestinal tract such as obesity, IBD, Type II diabetes and enteric infections, suggesting a correlation between the gut microbiota and health (Boulangé *et al*., 2016; Matsuoka & Kanai, 2015; Larsen *et al*., 2010; Singh *et al*., 2015). Probiotics are often recommended as a means of correcting imbalances in the gut microbiota. However, the introduction of these healthy microorganisms in order to improve health is not always effective, as they must firstly survive the acidic environment of the stomach and secondly compete with the natural flora of the large intestine in order to exert favourable effects. Therefore the use of prebiotics, indigestible food ingredients which stimulates the growth of one or a limited number of the natural flora in the intestine thereby conferring beneficial effects, should be considered.

Based on the three main criteria regarding prebiotics (non-digestibility, fermentative ability and selectivity), *in vitro* studies have identified several seaweed extracts as being rich in prebiotic potential. For example laminarin, the seaweed polysaccharide, has been shown to be resistant to hydrolysis by human digestive enzymes and was found to act as a modulator of intestinal metabolism through increased production of short chain fatty acids (SCFAs), lowering the intestinal pH (indicative of bacterial growth) and altering the mucus composition of animal models (Devillé *et al*., 2004; Devillé *et al*., 2007).

However, these studies suggest that while laminarin has a beneficial effect on the gut, the polysaccharide is not selectively fermented. When screening potential prebiotics, it is more desirable that the compound is fermented by the beneficial intestinal flora, such as *Lactobacillus* and *Bifidobacterium*, and not by potentially pathogenic strains in the gut (Figure 1.6).

While the prebiotic selectivity of laminarin has not been demonstrated, other seaweed extracts have been found to selectively promote growth of beneficial bacteria and inhibit the growth of pathogenic strains. Kong *et al*., (2016) found that sulphated polysaccharides from *L. japonica* and *E. prolifera* fermented by human faecal cultures significantly increased SCFA production and promoted the growth of *Lactobacillus* and *Bifidobacterium*. These prebiotic effects were reported to be linked to molecular weight of the sulphated polysaccharides. Similar studies have found that differences in physio-chemical properties of seaweed extracts have an influence on prebiotic activities which may be because some compounds are more susceptible to fermentation (Rodrigues *et al*., 2016; Ramnani *et al*., 2012). *In vivo* studies have found that supplementation with seaweed extracts improves growth performance of pig and reduces *E. coli* and *Enterobacteriaceae* populations in the gut (Leonard *et al*., 2011; Smith *et al*., 2011). It is clear that seaweed extracts are emerging sources of novel prebiotics both for human health and for use in the agricultural sector to improve growth performance of cattle.

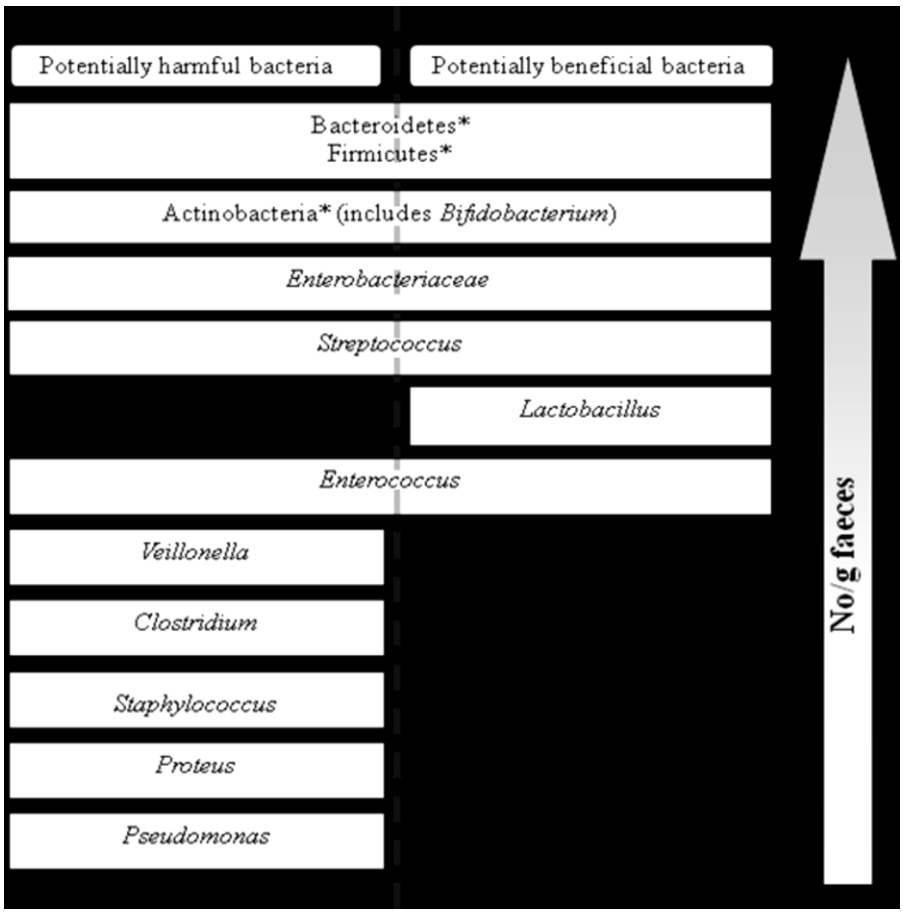


Figure 1.6: Distribution of the dominant, sub-dominant and minor components of human faecal microflora. *Major dominant phyla are denoted. Other components are at the family or genus level. (O'Sullivan *et al*, 2010)

5.2 Proteins

Global food requirements are constantly expanding along with global populations. However, some essential nutrient sources such as proteins have been predicted to be in short supply in the future, prompting industry driven research into novel and sustainable sources of proteins. Seaweed has been considered a viable source of protein. A study carried out on the brown macroalgae *Himanthalia elongata*, *Bifurcaria bifurcate* and *Laminaria saccharina* (also known as *Saccharina latissima*) determined that the protein content was within the range of 10.95-25.7% dry weight, with *Laminaria saccharina* containing the highest protein fraction (Gómez-Ordóñez *et al*., 2010). Protein content usually varies according to species and seasonal variation but is typically within the range of 3-47% dry weight, with red and green seaweed having higher protein content than brown seaweed (Fleurence *et al*., 1999).

Amino acid analysis of proteins isolated from 34 edible seaweeds was determined to be rich in all essential amino acids (EAA), particularly threonine, valine, leucine, lysine, glycine and alanine. In contrast to red macroalgae, levels of individual amino acids were found to vary between brown macroalgae species (Dawczynski *et al*., 2007). This pattern seemed to be displayed in other studies. A study on the nutritional composition of three brown macroalgae, *Padina pavonica*, *Dictyota dichotoma* and *Colpomenia sinuosa*, determined that total EAA levels in *P. pavonica* were suitable to meet the requirements set out by the WHO/FAO, whereas total EAA content in *D. dichotoma* and *C. sinuosa* were insufficient (Tabarsa *et al*., 2012). As the EAA profile is a common tool used to assess novel proteins, the total amino acid profile of the seaweed protein should be examined in order to determine its acceptability in human and animal nutrition.

However, digestibility of these proteins should be considered as a barrier towards the utilization of seaweed as a novel protein source. There are many exogenous and endogenous factors which may negatively affect digestibility of seaweed protein including species, seasonal variation and presences of various anti-nutritional compounds such as polysaccharides and phenolic compounds (Fleurence *et al*., 1999). Polysaccharides contained in the cell walls of seaweed, particularly brown seaweed, form stable complexes with proteins, which negatively influences protein digestibility. These polysaccharides often behave like soluble fibres which have been shown to reduce pepsin activity and thereby negatively influence protein digestibility (Horie *et al*., 1995). As a result, extraction methods such as enzyme hydrolysis, mechanical

grinding, ultra-sound assisted extraction and pulse electric field extraction have been utilised in order to improve algal protein bioavailability (Bleakley & Hayes, 2017).

5.3 Polyunsaturated Fatty Acids (PUFAs)

In general, seaweed has low total lipid content in comparison to other essential nutrients. Total lipid content within edible macroalgae is usually around 2% dry weight, with very little variation between red and brown species (Dawczynski et al. 2007) (Dawczynski et al., 2007). A high proportion of that lipid content is contributed by health promoting long chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and arachidonic acid. PUFAs are essential nutrients which cannot be synthesized in the body and therefore must be included in the diet. However, intake of PUFA's are generally insufficient to meet the recommended requirements set out by the FAO/WHO. A recent European survey on dietary fatty acid consumption determined that of the countries surveyed, only half met the recommended PUFA intake range of 6-11% total energy, with fats, oils and cereal products being the main contributing dietary factors (Eilander et al. 2015) (Eilander et al., 2015). Therefore novel, renewable sources of these bioactive are required in order to meet these recommendations in all countries.

PUFAs contain two classes of compounds: omega-3 fatty acids (n-3) such as α-linolenic acid (C18:3, n-3) and omega-6 fatty acids (n-6) such as linoleic acid (C18:2, n-6). These PUFAs act as precursors to long chain PUFAs which have important biological functions in the body. For example, the n-3 fatty acids EPA and DHA have been linked with proper foetal development, improved risk of cardiovascular disease and improved cognitive function in those suffering from mild Alzheimer's disease (Swanson *et al*., 2012). Higher levels of PUFAs have also been linked to improved gastrointestinal health through the reduction of obesity, improved insulin resistance and potent anti-inflammatory actions (Ruzickova *et al*., 2004; Albert *et al*., 2014; Monk *et al*., 2012). Seaweed lipids are ideal sources of essential fatty acids as in most cases the n-6: n-3 ratio is typically below 1 (Dawczynski *et al*., 2007; van Ginneken *et al*., 2011). The WHO have set out a recommended n-6/n-3 ratio of less than 10 in order to prevent the development of cardiovascular, inflammatory and neuronal disorders. However, Western diets are typically deficient in omega-3 fatty acids and therefore have an estimated n-6/n-3 ratio of approximately 15/1 to 16.7/1. The inclusion of low n-6/n-3 ratio seaweed products in Western diets could help alleviate many health concerns through dietary intervention.

5.4 Vitamins

Due to their habitat, seaweeds are often exposed to sunlight for long periods of time which stimulates the production of antioxidant compounds such as vitamins. As with other nutrients, vitamin levels often vary according to seaweed species, season of the year, salinity, sea temperature and light intensity. Seaweeds contain both water-soluble and lipid-soluble vitamins including, vitamin C, vitamin B, provitamin A and vitamin E, in levels usually sufficient to meet most recommended daily intakes for vitamins (Table 1.4). For example several edible seaweed such as *Macrocystis pyrifera, Ulva lactuca* and *Durvillaea antarctica* have been found to contain high levels of tocopherols when compared with traditional sources of tocols such as plant oils (Škrovánková, 2011; Ortiz *et al*., 2006)

Some seaweed species have been identified as potential sources of vitamin B12, which is not usually found in land plants. Vitamin B12 is a water soluble vitamin which works in conjunction with folate in the synthesis of DNA and red blood cells and is essential for the normal function of the brain and nervous system. Previous research has identified some algal species, as well as algal based food products, as sources of vitamin B12. However, the bioavailability of B12 from these sources varies. A diet of dried nori leaves were found to improve hepatic B12 levels in B12 deficient rats, which indicates its bioavailability in mammals (Takenaka *et al*., 2001). In contrast, spirulina tablets were found to be unsuitable as a source of B12 for mammals as pseudovitamin B12, a biologically inactive corrinoid, is predominant in spirulina tablets (Watanabe *et al*., 1999; Watanabe *et al*., 2002)

5.5 Minerals

Seaweeds are often considered concentrated sources of minerals due to their exposure to a wide variety of earth elements in their habitat, and their ability to concentrate these rare elements. As a result, these marine plants typically have a higher content of calcium, sodium, magnesium and potassium, along with trace minerals such as iodine, zinc and iron, when compared with terrestrial plants. For example, *Porphyra* spp and *Ulva lactuca* have a higher content of bioavailable iron when compared with spinach (Flores *et al*., 2015). This abundance of minerals has the potential to be utilized in the production of novel functional foods for health as the mineral content of seaweeds are usually sufficient to meet daily nutrient requirements (Table 1.4). One such example of an algal functional food is seaweed supplemented chocolate. This product was

developed as a means of improving iron intake in anaemic adolescent girls as haemoglobin levels, total iron binding capacity and serum iron levels were improved after dietary supplementation (Thahira Banu & Uma Mageswari, 2015). Another application of algae in the food and health industry is the development of algal mineral supplements. Aquamin F is a calcium and magnesium rich, multi-mineral alagal supplement, which has been linked with the treatment of osteoporosis through improved bone formation and reduced inflammation (O'Gorman, Tierney, *et al*., 2012; O'Gorman, O'Carroll, *et al*., 2012)

However, heavy metal pollution is a factor which can hinder the safety of seaweed as a food product. Inorganic arsenic, lead, mercury, copper and cadmium in particular are a concern, as chronic exposure to these heavy metals can lead to serious health risks. In Europe, there is little to no legislation focused solely on seaweed and seaweed containing products. Seaweed in general is considered as a novel food in Europe; however several edible macroalgae and microalgae species have been marked as not novel i.e. consumed to a substantial degree in the EU before May 1997 (Table 1.2). As such, these seaweeds are often classified under general regulations for food products (Table 1.3). However, legislation waivers for cadmium and lead were awarded as higher levels of these heavy metals occurs innately in macroalgae. A study was carried out to compare the heavy metal content in edible seaweed products to heavy metal legislation used in Spain. One hundred and twelve samples were assayed, including packaged seaweed products, canned seaweeds, seaweed tablets and extracts and food containing seaweed. A failure to comply with heavy metal legislation was observed in all assayed products, indicating a need for seaweed specific regulation (Almela *et al*., 2006). France was one of the first countries to assign specific regulations to seaweed based food products and has subsequently set out maximum heavy metal levels in seaweed destined for consumption (Table 1.4). Therefore, in order to ensure safety of consumers, clear and unified heavy metal limits for seaweed products should be put in place throughout Europe.

Table 1.2: Origin and species of edible seaweed which have been listed as not novel in EU Food Catalogue

Species Name	Origin of species
Ascophyllum nodosum	European
Eisenia bicyclis	SE Asian
Fucus vesiculosus	European
Hizikia fusiforme	SE Asian
Laminaria digitate	European
Laminaria longicruris	European
Palmaria palmata	European
Porphyra tenera	SE Asian
Saccharina japonica	SE Asian
Saccharina latissima	European
Undaria pinnatifida	European & SE Asian

Table 1.3: Maximum allowable heavy metal limits in France & tolerable weekly intake of heavy metals in Europe.

Heavy Metals	French Maximum Heavy Metal Limits* (mg/kg dry weight)	European Tolerable Weekly Intake of Heavy Metals** (mg/kg dry weight)
Inorganic Arsenic (As)	3	0.3-8
Cadmium (Cd)	0.5	7
Mercury (Hg)	0.1	1.6
Lead (Pb)	5	25°
Tin (Sn)	5	50-200
Iodine (I)	2000	100-150

* French heavy metal limits obtained from CEVA

** European tolerable weekly intake of heavy metals obtained from ''Commission (EC) No 1881/2006 - setting maximum levels for certain contaminants in foodstuffs'', ''Commission Regulation (EC) No 2015/1006'' and SCF (Scientific Committee for Food, 1993).

° Values expressed as µg/kg bodyweight

Table 1.4: Vitamin & mineral composition of selected seaweeds compared to European recommended daily intake.

Vitamin	**Seaweed**[1]				
	Palmaria palmata	***Ascophyllum nodosum***	***Saccharina latissima***	***Ulva sp***	***Recommended intakes***[2]
A	7.44	N/D[3]	99*	0.2	700-600**
E	3.4	14	0.6	1.95*	0.4mg x g dietary PUFA
K	0.42	1.017	N/D	N/D	65-80**
D	0.9	1	1	1.31	5**
C	83.6	94.8	11.3	54.6	45
B1	0.4	0.3	0.4	0.1	1-1.2
B2	0.5	1	0.3	0.3	1-1.3
B3	4.3	2.7	N/D	8.6	9-18
B9*	92	22.7	N/D	53	250-300**
B12*	9.8	2.1	N/D	9.6	0.6-1.4**
Minerals	**Seaweed**				
	Palmaria palmata	***Ascophyllum nodosum***	***Saccharina latissima***	***Ulva sp***	***Recommended intakes***
Sodium,	1659	2859	3590	1974	5000
Magnesium,	241	836	790	2776	150-500
Phosphorus,	280	162	230	181	550
Potassium	6812	2269	6180	1952	3100-3500
Calcium	547	1652	680	1198	1200-1300
Manganese	12.1	2.5	0.3	3.9	1-10
Iron	34.8	21.8	7.1	78.9	15-20
Copper	1.1	0.7	0.3	1.3	1.1
Zinc	4.2	6.4	2.5	3.7	7-9
Iodine	32.5	68.2	366	9.2	100-150**
Selenium*	9	6.7	N/D	14.9	55**

[1] Mean values of vitamin & mineral composition obtained from CEVA (Centre d'Etude et de Valorisation des Algues). Values expressed as mg/100g dry weight.
[2] Mean RI values obtained EFSA (European Food Safety Authority, 2006), SCF (Scientific Committee for Food, 1993) & World Health Organisation (WHO). Average RI's for adults expressed as mg/day.
[3] N/D = no data available
* Values expressed as µg/100g dry weight.
** RI's expressed as µg/day

6. Seaweed bioactives & gastrointestinal health

The gastrointestinal tract is a major contributor towards health as it facilitates the absorption of many essential nutrients and acts as a barrier to the external environment. The GI tract is one of the biggest components of the immune system as it contains effective detection systems for the presence of antigens and a large pool of immune cells available to mount the necessary response. However, disorders in these biological functions can lead to development of serious gastrointestinal disorders. A survey carried out on behalf of United European Gastroenterology determined an increase in the prevalence of gastrointestinal disorders such as inflammatory bowel disease (IBD), Celiac's disease and alcoholic liver disease, as well as colorectal and pancreatic cancers (Farthing *et al*., 2014). As such, effective preventative or amelioratory strategies must be developed in order lower the burden of future healthcare. A variety of marine bioactives have been found to exhibit anti-inflammatory, anti-tumour and anti-allergic properties, which may prove useful in the treatment of these gastrointestinal disorders (Islam *et al*., 2013; Kim *et al*., 2010; Sanjeewa *et al*., 2016). As the GI tract is the primary interface between the diet and essential biological processes, the incorporation of marine bioactives in functional food or pharmaceuticals may have a role in treating and preventing these disorders.

6.1 Anti-inflammatory activities

Inflammation is a complex biological response to harmful stimuli such as pathogens and cell injury. It utilises immune cells, blood vessels and biological mediators as a means of removing these harmful stimuli. However, chronic inflammation is detrimental to cells and can often lead to the pathogenesis of inflammation-derived diseases such as gastrointestinal cancers, atherosclerosis and inflammatory bowel disease (IBD) (Macarthur *et al*., 2004; Libby *et al*., 2002). IBD is a blanket term used to describe chronic inflammatory conditions which affects all or part of the gastrointestinal tract. IBD typically describes two conditions: Crohn's disease, which affects the entire GI tract, and ulcerative colitis, which only affects the colon. Although the exact aetiology and pathogenesis of IBD is unknown, a combination of genetic susceptibility and environmental factors has been linked to the initiation and progression of this inflammatory disorder (Figure 1.7).

IBD is a global disease, with highest incidence rates being reported in industrialized regions such as Canada and Northern Europe (Molodecky *et al*., 2012). Previously low

incidence regions, such as Asia, have also experienced a dramatic increase in incidence of IBD, which has been associated with rapid urbanization and therefore exposure to associated environmental factors such as decreased physical activity and a more ‘’Westernized’’ diet (Yang *et al*., 2016). Younger populations in urbanized societies are also becoming more affected by IBD. A study in northern France found that between the years 1988-2007, incidence of Crohn’s disease increased by 71% in a group aged 10-19 years (Chouraki *et al*., 2011). Due to the increasing incidences of IBD worldwide, an economic burden will be placed on the healthcare system as patients diagnosed with IBD for less than 5 years have been found to have more frequent emergency room visits, hospitalizations and hospitalizations, followed by surgery than the general population (Longobardi *et al*., 2004).

Seaweed and seaweed extracts, particularly those from brown seaweed, have potent anti-inflammatory and immunomodulatory properties, which could be utilised in the treatment of inflammatory disorders such as IBD. These anti-inflammatory properties have largely been tested within in vitro or animal models. Several in vitro studies have determined the inhibitory effect of the marine carotenoid fucoxanthin on inflammatory mediators and pro-inflammatory cytokines using lipopolysaccharide stimulated RAW 264.7 macrophages. These studies determined that fucoxanthin inhibited nitric oxide and prostaglandin E2 production through the downregulation of inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) protein and mRNA expression (Shiratori *et al*., 2005; Heo *et al.*, 2010; Heo *et al*., 2012). Fucoxanthin has also been found to have an inhibitory effect on the release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) (Heo *et al*., 2012). Much of these anti-inflammatory activities are attributable to fucoxanthin’s ability to reduce nuclear factor-κB (NF-κB) activity and prevent mitogen-activated protein kinase (MAPK) phosphorylation (K. N. Kim *et al*., 2010). A number of other macroalgae-derived bioactives have also shown potent anti-inflammatory activities similar to fucoxanthin in different in vitro studies including polysaccharides such as fucoidan, algal lipids, and polyphenols such as phlorotannins as well as other aqueous extracts (Park *et al*., 2011; Robertson *et al*., 2015; Wijesinghe *et al*., 2013; Khan *et al*., 2008).

The potential advantages of seaweed and seaweed extracts as a nutraceutical in the treatment and management of inflammatory GI disorders has largely been exhibited by animal models. Two different orally administered preparations of fucoidan from the

brown macroalgae F. vesiculosus were found to ameliorate symptoms of colitis in murine models through the retention of body weight, reduction of diarrhoea and the decreased production of several pro-inflammatory cytokines by colonic tissue (Lean *et al*., 2015). Similar observations were found in dextran sodium sulphate (DSS) challenged porcine models after oral administration of algal polysaccharides laminarin and fucoidan (O'Shea *et al*., 2016). As there are little to no in-depth human trials, further research in humans is necessary to fully explore the anti-inflammatory activities of macroalgae compounds and their potential in the treatment of IBD.

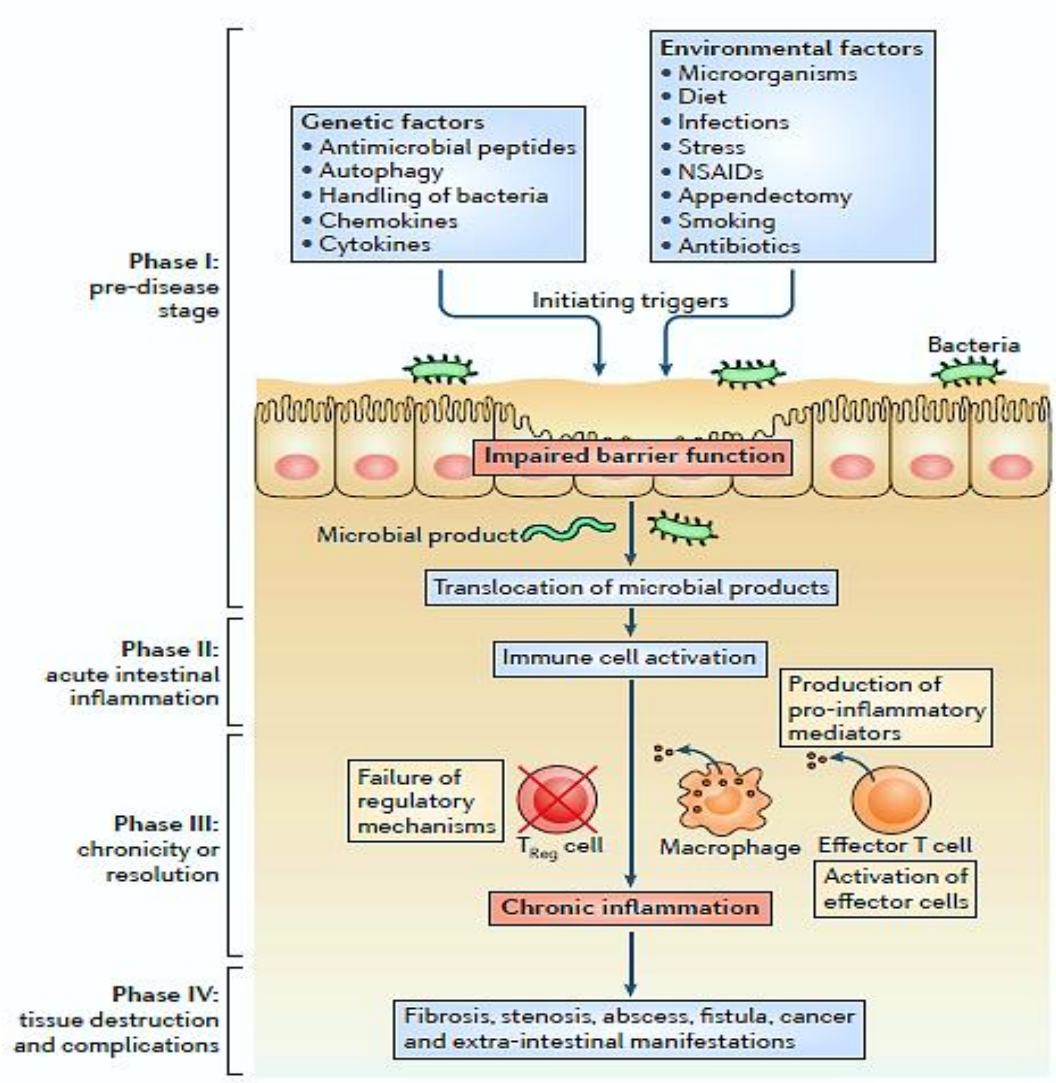


Figure 1.7: *Pathogenesis of IBD. Genetic and environmental factors induce impaired barrier function which allows translocation of microbial products into the bowel wall. Detection of microbial products induces immune cell activation and release of pro-inflammatory cytokines thereby causing acute inflammation. If regulatory mechanisms fail to resolve mucosal inflammation, chronic intestinal inflammation occurs. Chronic inflammation can lead to tissue destruction and induction of gastrointestinal disease. (Adapted from Neurath, 2014)*

6.2 Anti-obesity/weight management

Obesity is one of the world's most visible yet neglected health concerns. It can be defined as an abnormal or excessive accumulation of fat, which can present a risk to health. According to WHO, 13% of the global population are obese, with 11% of men and 15% of women found to be obese in 2014 (WHO, 2016). However, global obesity rates are on the rise and, according to a recent study published in the Lancet, by 2025, global obesity prevalence will reach 18% for men and surpass 21% for women (Ng *et al*., 2014; Di Cesare *et al*., 2016). This presents a serious global health challenge, as obesity is often associated with increased risk of developing other disorders, including Type 2 diabetes, hypertension, metabolic syndrome, osteoarthritis and even some forms of cancer. Management of body weight is one of the methods used in obesity treatments. This is usually achieved through a permanent change in diet, food intake and level of physical activity. In some extreme cases, anti-obesity drugs may be prescribed. These pharmaceuticals treat obesity through a reduction in energy absorption or by reducing fat mass, either through an increase in energy expenditure or by redistributing adipose tissue. Currently, there are only a few anti-obesity drugs on the commercial market, with many more undergoing clinical and pre-clinical trials. As such, there is an increasing demand for more anti-obesity compounds, particularly from natural sources.

Potential therapeutic benefits of seaweed consumption have been reported in the management of obesity. A recent study evaluated the effect of seaweed powder obtained from *Sargassum polycystum* on rats fed with a high fat diet. Supressed weight gain was evident in all groups and positive reductions in plasma levels of cholesterol and triglycerides were observed in the high dosage group (Awang *et al*., 2013). This may be due to the high fibre content in the seaweed species, as high fibre diets have been found to promote weight management through delayed gastric emptying and improved post-meal satiety. Seaweed is a rich source of dietary fibres. One such example would be alginate, a soluble dietary fibre which can be found in the cell wall of brown seaweed and is often used in the food industry as an emulsifiers and stabilizers. Alginates have also been added to drink formulations as a means of enhancing post-meal suppression of hunger, although the reduction in hunger response depends on the gastric gelling ability of the alginate used (Peters *et al*., 2011). As such, several studies have investigated the potential benefits of alginates extracted from brown seaweed in weight management. Jensen *et al*., (2012) investigated the effect of alginate supplementation on the weight loss of obese subjects on an energy restricted diet for 12 weeks. A greater degree of

weight loss were observed in those given the alginate supplement, when compared to the placebo group, which suggests that alginate supplementation may improve weight loss in subjects undergoing dietary intervention treatments.

Phlorotannins, fucoidan and fucoxanthin have all been identified as potential anti-obesity agents. Phlorotannins have been shown to hinder adipocyte differentiation, a strategy which could be used in the prevention and treatment of obesity. Obesity is associated with increased proportions of adipose tissue, which can be regulated through the suppression of adipogenesis. Phlorotannins fractions isolated from the brown macroalgae *Ecklonia stolonifera* have been found to diminish expression of adipocyte gene markers such as proliferator activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα) (Jung *et al*., 2014). These adipokines play vital roles in the development of fat cells and PPARγ, in particular is highly expressed in adipose tissue. Fucoidan, fucoxanthin and fucoxanthin's metabolite fucoxanthinaol were likewise found to supress adipocyte differentiation, through the downregulation of PPARγ, with fucoxanthinol exhibiting stronger suppressive effects than fucoxanthin (K. J. Kim *et al*., 2010; Maeda *et al*., 2006).

One of the main causes for the increasing interest in brown seaweed and its derivatives as anti-obesity agents is due to their inhibitory role against pancreatic lipase. The inhibition of lipases, specifically pancreatic lipase, is a major target for most anti-obesity drugs as the digestion and absorption of dietary lipids by pancreatic lipase causes an excess of calorie intake. Orlistat, a commercially available anti-obesity drug, is a potent inhibitor of gastric and pancreatic lipases. This compound inactivates the lipases by forming covalent bonds with the active sites of the lipases. However, adverse side effects such as diarrhoea, abdominal cramping and deficiencies in fat-soluble vitamins limit its value to patients (Lunagariya et al., 2014). Therefore, anti-lipase compounds from natural sources with little to no adverse side-effects are required. Preparation from three different brown seaweeds, A. nodosum, F. vesiculosus, and Pelvetia canaliculata were tested for anti-lipase activity. The preparations tested, which included whole seaweed homogenate, sodium carbonate extracts and ethanol extracts, all demonstrated significant lipase inhibition (Chater *et al*., 2016). This implies numerous biologically active agents present in the seaweed, which could be utilised for anti-obesity treatments. These bioactive agents may include alginates, polyphenols and fucoxanthin, which have all been marked for anti-pancreatic lipase activity in previous studies (Houghton et al., 2015; Buchholz & Melzig, 2015; Matsumoto *et al*., 2010).

6.3 Anti-diabetic

Non-insulin dependent diabetes or Type II diabetes is a chronic metabolic disorder which is characterized by increased insulin resistance, high blood glucose levels and reduced production of insulin. There are several risk factors which can contribute to the development of type II diabetes including genetic predisposition, lifestyle factors such as smoking and physical activity and deficiencies in essential vitamins such as vitamin D (Wu *et al*., 2014). However, type II diabetes and its precursor of insulin resistance is usually a consequence of prolonged obesity. During obesity, increased release of factors such as hormones, non-esterified fatty acids and pro-inflammatory cytokines contributes to the development of insulin resistance. This insulin resistance, paired with aberrations in pancreatic beta-cell function, results in an inability to control blood glucose levels, thereby contributing to development of type II diabetes (Kahn *et al*., 2006). Type II diabetes is a visible global epidemic. In 2013, approximately 382 million people were estimated to have diabetes, with that number expected to rise to 592 million in 2035 (Guariguata *et al*., 2014). Therefore effective strategies to improve insulin sensitivity and prevent development of type II diabetes are required.

Promising anti-diabetic effects have been identified from consumption and supplementation with seaweed. A national survey carried out in Korea found that dietary consumption of seaweed is associated with reduced risk of type II diabetes in Korean men, a statement which has been illustrated in various animal and clinical studies (Lee *et al*., 2010). Selvaraj & Palanisamy, (2014) observed potent hypoglycaemic effects in alloxan-induced diabetic rats after consumption of brown macroalgae *Sargassum longiotom* extracts. When compared with untreated diabetic rats, rats administered these extracts exhibited a significant reduction in blood glucose levels. The influence of seaweed on glycaemic control was exhibited in a clinical study involving patients with type 2 diabetes. Patients were supplemented with tablets composed of equal parts sea tangle (*Laminaria japonica*) and sea mustard (Wakame), three times a day for approximately 4 weeks. Fasting blood glucose levels and postprandial blood glucose levels were reduced significantly in the seaweed supplementation group. This reduction has been linked to increased fibre content, as those ingesting seaweed had a 2.5 higher fibre intake (Kim *et al.*, 2008). As seaweed is a rich source of fibre, which has been linked with improved glycaemic control in diabetic patients, the inclusion of seaweed supplement in the diet of diabetic patients may contribute to improved blood glucose levels.

Several specific seaweed extracts have been associated with potent anti-diabetic activities. Phenolic rich compounds isolated from brown seaweed in particular, have been examined in several studies against a variety of anti-diabetic targets. These targets include enzymes involved in glucose homeostasis, such as α-amylase and α-glucosidase, uptake of glucose by cells through various mechanisms and release of incretin hormones (Sharifuddin *et al*., 2015; Lopes *et al*., 2016). Dietary starch is a major source of glucose in the diet and increase in post-prandial blood glucose concentrations are typically caused by the hydrolysis of carbohydrates by α-amylase and α-glucosidase. These are significant enzymes in the breakdown and absorption of carbohydrates and inhibition of α-amylase and α-glucosidase are key targets in many anti-diabetic treatments. Lordan *et al*., (2013) compared 15 native Irish seaweed species for α-amylase and α-glucosidase inhibitory activities. Of those assessed, 5 seaweed species including *Ascophyllum nodosum, Fucus serratus, Fucus spiralis, Fucus vesiculosus* and *Pelvetia canaliculata*, were found to be strong inhibitors of α-amylase and α-glucosidase activity in levels well below cytotoxicity levels. *A. nodosum* and *F. vesiculosus* in particular, were found to be potent inhibitors of α-amylase and α-glucosidase, respectively, with much of these inhibitory activities associated with phenolic content and antioxidant activities of the seaweed species. Inhibitory activities of *A. nodosum* and *F.* vesiculosus extracts against α-amylase and α-glucosidase have been expressed in several other studies. Kim *et al*., (2014) found that fucoidan isolated from *A. nodosum* inhibited both α-amylase and α-glucosidase, while fucoidan from *F. vesiculosus* only inhibited α-glucosidase, which indicates their potential for diabetes management.

Several in vitro studies have illustrated the ability of brown seaweed extracts to promote incretin hormone secretion and thereby improve insulin secretion. A study of selected Malaysian seaweeds found that crude water extracts of three brown seaweeds, *Padina sulcata, Sargassum binderi* and *Turbinaria conoides*, stimulated glucose-dependent insulinotrophic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) secretion in an endocrine cell line and inhibited the production of dipeptidyl-peptidase-4 (DPP-4) (Chin *et al*., 2015). GIP and GLP-1 are gut-derived incretin hormones which promote the secretion of insulin in a glucose-dependant manner. These incretin hormones are rapidly hydrolysed by the enzyme DPP-4, which circulates in the body. Therefore, secretion of incretin hormones and DPP-4 inhibition are key anti-diabetic targets. Some seaweed extracts have been found to possess a similar efficacy to well-known anti-diabetic drugs

in promoting insulin secretion. A study compared the ability of water, ethanol and acetone extracts of the brown seaweed *Sargassum hemiphyllum* to stimulate insulin secretion with that of the known anti-diabetic drug glibenclamide. The authors found that all extracts stimulated insulin secretion, with the acetone extracts exhibiting similar efficacy to glibenclamide, which was linked to the high content of polyphenol and fucoxanthin content in the acetone extracts (Hwang *et al*., 2015). During a co-treatment of extracts and glibenclamide, it was found that insulin secretion was increased to a higher degree than with glibenclamide alone, with little to no increase in adverse side effects. This indicates the suitability of seaweed extracts as not only an anti-diabetic drugs but as a compound which could enhance the function of already available anti-diabetic drugs.

6.4 Anti-tumour

Cancers are a group of diseases characterized by uncontrolled division of abnormal cells in the body. Colorectal cancer is the third most common cancer worldwide, with more than 300,000 new cases reported in Europe in 2012 (Ferlay *et al*., 2015) (Figure 1.8). Although colorectal cancer death rates have decreased by 50% from peak rates, due to improvements in early detection methods and treatment, colorectal cancer has a high mortality rate linked to its tendency to metastasize (Siegel *et al*., 2016) (Figure 1.9). Due to the rising incidence of cancer, there has been increased interest in chemotherapeutic molecules from natural sources as a means of slowing or preventing the progression of invasive cancers (Nobili *et al*., 2009). Seaweed has several compounds which could prove useful in the treatment of colorectal cancer. (Hoshiyama *et al*., 1993) found that seaweed consumption is inversely related to the risk of developing colon and rectal cancers, through a dose-dependent relationship. A recent animal study investigated the effect of sea mustard (*Laminaria japonica*) and sea tangle (*Undaria pinnatifida*) consumption on the initiation of colon and liver carcinogenesis in mouse models. The study determined that consumption of extracts from these seaweed species may prevent the development of colon and liver cancer by inhibiting DNA damage related to the initiation of cancer (Bu *et al*., 2014). As such, an abundance of in vitro and in vivo studies have been carried out to elucidate the anti-cancer properties of various seaweed species.

A variety of bioactive compounds, including fucoxanthin and fucoidan have been attributed to the anti-cancer properties in seaweed. These compounds utilise several therapeutic targets in the suppression of cancer. For example, induction of apoptosis is a

popular target in many anti-cancer treatments. Apoptosis is a form of programmed cell death and defective expression of apoptosis has been reported as a causative factor in development of cancer. Fucoxanthin from the brown seaweed *Undaria pinnatifia* was found to reduce cell viability in several colon cancer cell lines, as well as induce DNA fragmentation, which is an indicator of apoptosis (Hosokawa *et al*., 2004). Fucoxanthin was also found to supress expression of Bcl-2, a protein with inhibitory influence on apoptosis. The inhibitory effect of fucoxanthin on the proliferation of colon cancer cells can be linked to its ability to induce cell cycle arrest at the G0/G1 phase, through the up-regulation of the cell cycle regulator p21 (Das *et al*., 2005). Fucoxanthin has the potential to prevent the development of colorectal cancer initiated by prior conditions, such as IBD and ulcerative colitis. Both of these conditions have been associated with increased risk of developing colorectal cancer. An analysis of population based cohort studies determined that those with ulcerative colitis had an increased risk of developing colorectal cancer (Jess *et al*., 2012). Z. Kong *et al*., (2016) found that not only did fucoxanthin reduce the inflammatory response of mouse models with DSS-induced colitis, but also decreased the incidence of colonic neoplasm and increased the rate of survival in colon associated colorectal cancer (CACC) mice.

Similar to fucoxanthin, studies have elucidated the capability of fucoidan to reduce cell viability through the induction of apoptosis in several colon cancer cell lines (Hyun *et al*., 2009; E. J. Kim *et al*., 2010). Other anti-carcinogenic properties of fucoidan include the prevention of the invasion, metastasis and angiogenesis of cancer cells through the inhibition of growth signal mechanisms. Metastasis, the development of secondary malignant growth at a different location from the original site of cancer, is one on the leading causes of cancer deaths and as such is a significant target in cancer treatments. Using a hepatocarcinoma cell line, fucoidan isolated from *Undaria pinnafidia* sporophylls were found to inhibit tumour metastasis in vitro in a concentration and time-dependant manner and prevent the growth, invasion and adhesion abilities of the cell line in vivo (Wang *et al*., 2014). These inhibitory actions were mediated by the down-regulation of PI3K/Akt and ERK signalling pathways, which are often altered during cancer thereby promoting metastasis.

The anti-angiogenic properties of fucoidan have also been expressed in the literature. Angiogenesis is the formation of new blood vessels, which is vital for wound healing and embryonic development. During cancer, unregulated angiogenesis can contribute to tumour progression. A fucoidan fraction isolated from *Sargassum fusiforme* dose

dependently inhibited migration and tube formation of human microvascular endothelial cells, which indicates its suitability as a potent anti-angiogenic mediator. However, molecular weight and sulphate content of the fucoidan fraction is an important factor in its anti-angiogenic efficacy. For example, a study compared the anti-tumour and anti-angiogenic properties of over-sulphated fucoidan to normal fucoidan. It was determined that suppressive effect of over-sulphated fucoidan on the angiogenic growth factor, vascular endothelial growth factor 165 (VEGF165) was greater than in normal fucoidan fractions (Koyanagi *et al*., 2003). This indicates that the addition of sulphate groups to fucoidan fractions may improve the efficacy of fucoidan as an anti-cancer agent.

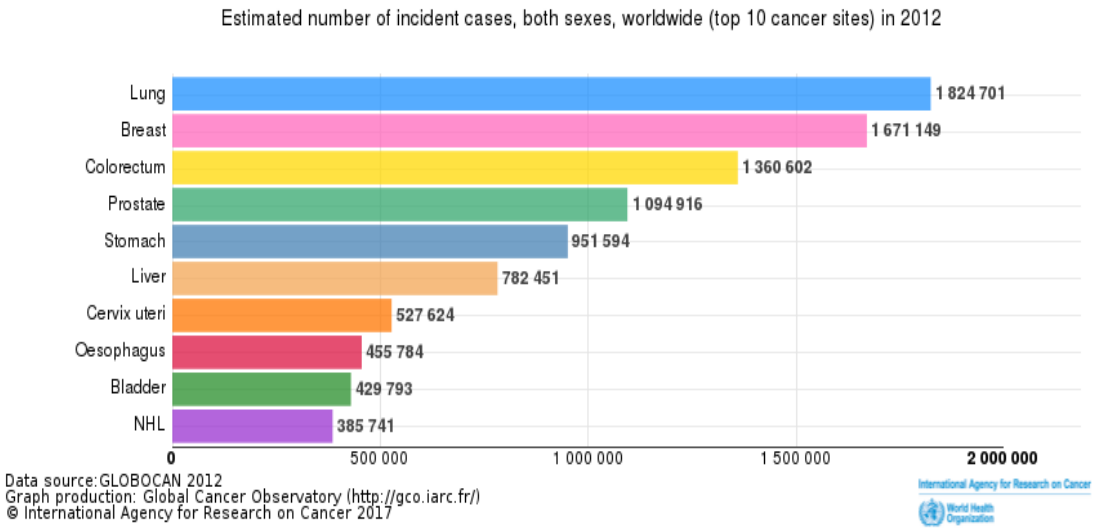


Figure 1.8: *Estimated incidence of cancers worldwide in 2012. Lung cancer demonstrated highest incidence in 2012, followed by breast cancer and colorectum cancer*

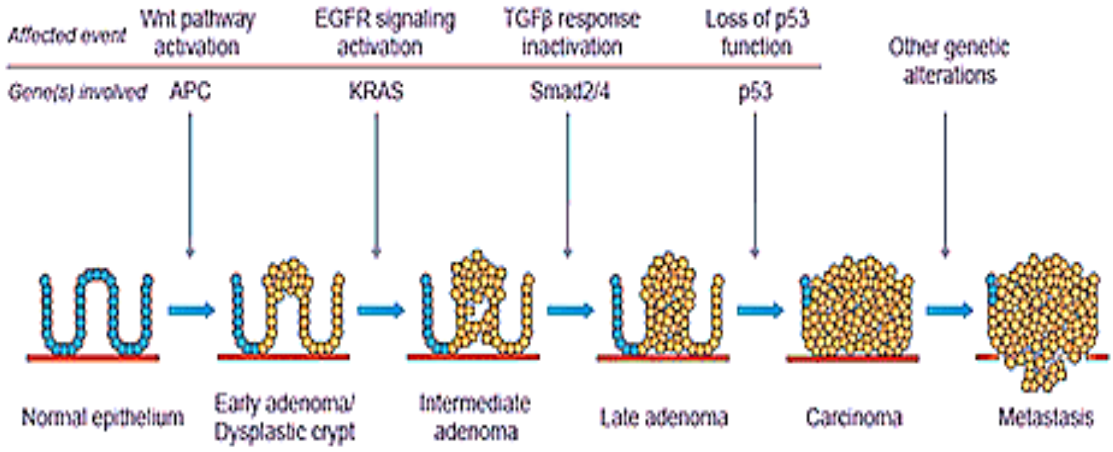


Figure 1.9: *Progression of colorectal cancer from normal epithelium and the main genes involved*

7. Conclusion

The utilization of marine resources such as seaweed as a source of bioactive compounds is still a relatively novel concept outside Asia. In many European countries the seaweed industry itself is just starting out. With the rising global population and with it, rising demands for food and resources, it is clear that the exploitation of untapped marine sources is the way forward. Seaweeds, in particular brown seaweed, are compelling sources of nutrients and novel bioactive compounds which has implications for many chronic non-communicable diseases of the gastrointestinal tract, such as inflammatory bowel disorder (IBD), colorectal cancer, type II diabetes and obesity. An abundance of *in vitro* and *in vivo* trials provide the majority of data on the health benefits of these marine bioactives and in order to further the development of marine bioactives into functional nutraceuticals and even pharmaceuticals, reliable human data must be achieved through clinical trials.

Abstract

Due to rapid global urbanization and therefore increased adoption of a ‘’Westernized’’ lifestyle, including decreased physical activity and increased consumption of highly processed and refined foods with a high sugar, fat and salt content, prevalence of gastrointestinal disorders are on the rise. Inflammatory bowel disease (IBD) in particular is closely associated with this type of lifestyle. IBD is a term used to describe chronic inflammatory conditions which affects all or parts of the gastrointestinal tract. It includes conditions such as Crohn’s disease, which affects all the gastrointestinal tract and ulcerative colitis which mainly affects the colon. Due to the role of chronic inflammation in the development of gastrointestinal malignancies, development of these disorders has also been linked to increased risk of colorectal cancer.

While the exact aetiology of IBD remains unclear, key features of this disease have been identified as therapeutic targets, such as abnormal immune responses. As such current therapeutic methods are aimed at the suppression of these immune responses. Along with the mentioned environmental factors, dysregulation of the gut microbiota has been linked to the pathogenesis of IBD. The gastrointestinal tract host a complex community of microorganisms which are integral to host’s health. Microbial dysbiosis is a common symptom associated with IBD and is thought to contribute to the chronic inflammatory responses observed in this disorder. However, use of immune suppressing agents to treat IBD may increase susceptibility to foodborne or hospital infections.

With incidences of IBD increasing, novel bioactives from natural sources have been considered as a means to manage this disorder. Seaweed and seaweed extracts, particularly those from brown seaweed, have potent anti-inflammatory and anti-microbial properties which could be utilised in the treatment of IBD. Seaweed has also been noted as a potential source of prebiotics, which could promote a balanced microbial community in the gut. The aim of this project was to assess the anti-inflammatory properties of extracts from four brown seaweed species *Saccharina latissima*, *Alaria esculenta*, *Ascophyllum nodosum* and *Fucus vesiculosus* using an *in-vitro* model of gastrointestinal inflammation

Aims of Thesis

- To assess the anti-inflammatory properties of extracts from four brown seaweeds : *Saccharina latissima*, *Alaria esculenta*, *Ascophyllum nodosum* and *Fucus vesiculosus* by determining potential inhibitory activities against IL-8 production

- To further assess the anti-inflammatory of the seaweed extracts using an *in-vitro* model of gastrointestinal inflammation

- To determine the anti-microbial properties of *Saccharina latissima*, *Alaria esculenta* and *Ascophyllum nodosum* extracts against a number of bacteria including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Salmonella enterica*.

- To determine whether antimicrobial properties of the seaweed extracts are bactericidal or bacteriostatic

- To investigate potential detrimental effects of seaweed extracts on growth kinetics of probiotic strain *Lactobacillus johnsonii*.

Chapter 2

Determination of anti-inflammatory properties of brown seaweed extracts using *in vitro* models of gastrointestinal inflammation

1. Introduction

Inflammatory bowel disease (IBD) is a term used to describe chronic inflammatory conditions which affects all or parts of the gastrointestinal tract, for example Crohn's disease and ulcerative colitis. IBD is a global disease, with the highest incidences reported in industrialized areas such as Canada and Northern Europe (Molodecky *et al*., 2012). Development of these disorders has been linked to increased risk of colorectal cancer, as chronic inflammation can cause the development of gastrointestinal malignancies. With incidences of gastrointestinal disorders increasing across Europe, as well as increased prevalence of IBD in previously low incidence areas, an economic burden will be placed on the global healthcare system prompting the need for novel therapeutics for the management of this condition.

While the exact aetiology of IBD remains unclear, understanding of the pathophysiology of IBD has advanced, with many features of the disease acting as key therapeutic targets. For example, compromised intestinal barrier function has been identified as a key feature of IBD. In normal physiology the intestinal barrier is a complex system formed by intestinal epithelial cells which has roles in the production and regulation of mucus, controlling antigen passage by acting as a physical barrier and interacting with the cells of the intestinal immune system. During IBD, barrier function is disrupted causing increased permeability, reduced numbers of secretory cells, impaired tight junctions, loss of epithelium due to the formation of ulcers and increased passage of bacterial and dietary antigens, thereby causing increased activation of mucosal immune cells. It has been suggested that impaired barrier function in IBD is a consequence of increased mucosal inflammation, another common therapeutic target in the treatment of this disorder.

Abnormal immune responses to intestinal microbes and ingested substances are a common feature of IBD. During normal inflammatory conditions, once inflammatory stimuli are eliminated, pro-inflammatory responses typically shift to anti-inflammatory responses thereby downregulating the inflammation process. However, due to defects in the function of intestinal immune cells, shifts from pro-inflammatory to anti-inflammatory responses are impaired in IBD, leading to chronic inflammation in the gastrointestinal tract. Many of these inflammatory responses are mediated by pro-inflammatory cytokines and chemokines such as IL-6, TNF-α, MCP-1 and IL-8 (Figure 2.1). Interleukin 8 is a chemotactic cytokine which plays a role in the pathophysiology

of many diseases through the promotion of leukocyte migration to areas of inflammation and the initiation of cell activation events. Several studies have found that IL-8, along with other cytokine such as IL-6 and TNF-α, is highly expressed in patients with inflammatory bowel disease and other forms of colitis (McCormack *et al*., 2001; Mitsuyama *et al*., 1994). As such suppression of pro-inflammatory cytokine release has been identified as a therapeutic target for the treatment of these inflammatory disorders.

Current treatment methods include the use of non-biological therapies such as steroids and aminosalicylates. However, while the therapeutics provides relief from symptoms of IBD progression of the disease is unchanged. In the case of severe IBD, this can lead to surgery. The introduction of anti-TNF agents such as infliximab has reduced the need for surgeries and improved quality of life for patients by changing the progression of the disease (Hanauer *et al*., 2002). However, not all patients respond to treatments and these agents are associated with adverse side effects, including risks of infections and development of extra-intestinal malignancies (Ford & Peyrin-Biroulet, 2013; Axelrad *et al*., 2016). As a result, there has been increasing interest in alternative methods, such as the use of nutraceuticals and bioactive dietary components, to treat IBD (Larussa *et al*., 2017; Zhang *et al*., 2015).

Seaweed and seaweed extracts, particularly those from brown seaweed, have been highlighted due to their rich bioactive potential. Extracts from brown seaweed has been found to possess many bioactive properties, including anti-oxidant, anti-thrombotic, anti-obesity, and anti-diabetic properties (O'Sullivan *et al*., 2011; Zhao *et al*., 2012; Wan-Loy & Siew-Moi, 2016; Chin *et al*., 2015). Brown seaweed extracts have also been found to possess potent anti-inflammatory and immunomodulatory properties which could be utilised in the treatment of gastrointestinal inflammatory disorders. For example *in-vitro* testing has shown that seaweed extracts reduce nitric oxide and prostaglandin E_2 production, supress the expression of pro-inflammatory genes, reduce pro-inflammatory cytokine levels such as IL-1β, IL-6 and TNF-α and promote anti-inflammatory cytokines IL-10 and IFN-y. Intake of brown seaweed extracts fucoidan, laminarin and fucoxanthin has also been found to reduce inflammatory pathology in animal models with induced colitis (Lean *et al*., 2015; O'Shea *et al*., 2016; Kong *et al*., 2016). The objective of this study was to determine the anti-inflammatory properties of extracts from three different species of brown seaweed; *Saccharina latissima*, *Ascophyllum nodosum Alaria esculenta* and *Fucus vesiculosus* using an *in-vitro* model

of gastrointestinal inflammation. The potential inhibitory effect of these extracts on IL-8 production was investigated.

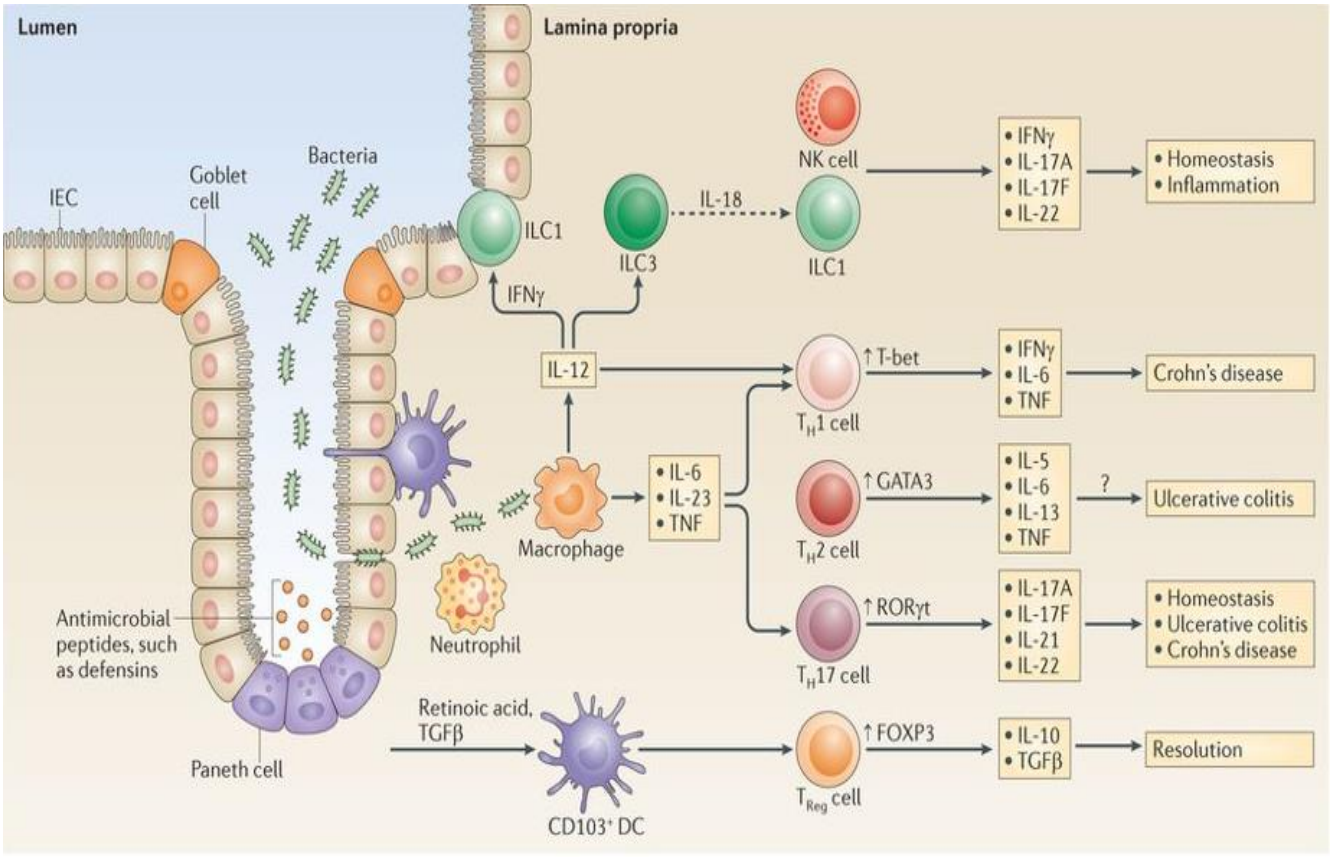


Figure 2.1: *Cytokines in the pathogenesis of IBD. During IBD, barrier function is disrupted, causing increased permeability and increased passage of bacterial and dietary antigens. Presence of these antigens promote the release of pro-inflammatory cytokines by immune cells. Adapted from Neurath et al., (2014).*

2. Material & Methods

2.1 Seaweed materials

Extracts from brown macroalgae species *Saccharina latissima*, *Ascophyllum nodosum*, *Alaria esculenta* and *Fucus vesiculosus* were provided by SeaRefinery partners Cybercolloids and Marinox (Table 2.1). The brown macroalgae were harvested from different locations (Ireland, Denmark and Scotland)(Table 2.1). *Saccharina latissima* and *Alaria esculunta* extracts were isolated by means of water extraction (Table 2.1). *Ascophyllum nodosum* extracts from different locations and different seasons were isolated by means of methanol, ethanol or water extraction (Table 2.1). Dried extracts were dissolved in phosphate buffer saline solution (PBS).

2.2 Cell Culture

Colon epithelial cells, CaCo-2 (obtained from EATCC), were maintained in Minimum Essential Medium Eagle (MEM), supplemented with 10% heat inactivated foetal bovine serum (FBS), 1% non-essential amino acids (NEAA), 2mM L-glutamine and 2mM penicillin/streptomycin. Murine macrophage cells, J774.2 (obtained from EATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% FBS, 2mM L-glutamine and 2mM penicillin/streptomycin. All cells were incubated at 37°C in a 5% CO_2, 95% air and humidified atmosphere, in a SANYO CO_2 incubator (Model number: MCO-15AC). Frozen cell stocks were maintained at -80°C, in complete medium and 20% dimethyl sulfoxide (DMSO). CaCo-2 cells used were in passages numbers 43-48 and J774.2 cells were used in passages numbers 7-30.

2.2.1 Cell Sub-culture

Both CaCo-2 cells and J774.2 cells are adherent cells. Medium was changed every 2-3 days until 70-80% confluence was achieved. Once confluent, cells were sub-cultured. To form a single cell suspension prior to sub-culturing, cells were washed with PBS and incubated with 0.25% Trypsin, 0.2% EDTA solution at 37°C for 2-3 minutes or until cells had detached from the flask. Trypsin activity was deactivated by the addition of equal volumes of complete medium. Cell suspension was pelleted by centrifugation at 1,000 rpm for 4 minutes. The cell pellet was re-suspended in fresh medium and cell numbers were enumerated. Cell suspension was either seeded at appropriate densities for experiment or seeded in new cell culture flasks. Viable cell counts were obtained using Trypan Blue and a haemocytometer.

2.3 Cytotoxicity Testing

Cytotoxicity of seaweed extracts on CaCo-2 and J774.2 cell lines were investigated using the Neutral Red uptake assay as described by Repetto et al., 2008. Cells were seeded at 15x104 cells per well in 96 well plates and allowed to adhere overnight. Cells were then treated with serial dilutions of seaweed extracts (200mg/ml – 1.5625mg/ml) for 24 hours. Extracts were decanted and cells were incubated at 37°C for 2 hours in the presence of neutral red working medium (neutral red dye diluted 1:100 in serum free DMEM). Neutral red medium was removed, cells were washed with PBS and neutral red de-stain solution (50% ethanol 96%, 49% deionized water & 1% glacial acetic acid) was added. Optical density of extracted dye was read at 540nm using a spectrophotometer. Experiments were carried out in triplicate.

2.4 Anti-inflammatory Screening

CaCo-2 cells were seeded at $1x10^6$ cells per well in 6 well plates or $2x10^5$ cells per well in 24 well plates and allowed to grow to confluence. Once confluent, cells were treated with serum free media (negative control), 1ug/ml lipopolysaccharide (LPS) (positive control) or dilutions of seaweed extracts (200mg/ml – 3.125mg/ml) plus 1ug/ml LPS for 24 hours. Extracts used for anti-inflammatory testing were chosen based on cytotoxicity results, origin of species, harvesting time and, in the case of *Ascophyllum* extracts, polyphenol content. Cell supernatants were collected and stored at -20°C for further analysis using ELISA's. Treated cells were stored at -80°C for use in RNA extraction and RT-PCR. Experiments were carried out in triplicate

2.5 Co-culture Set-up

An *in-vitro* model of gastrointestinal inflammation was set up as per Tanoue et al., 2008 with minor adjustments (Figure 2.2). CaCo-2 cells were seeded at $2x10^5$ cells per well onto 6 well Transwell inserts or $2x10^4$ cells per well onto 12 well Transwell inserts. The culture medium was changed every 2-3 days. Transwells were assessed visually until cells were fully differentiated. J774.2 cells were seeded at $8x10^5$ cells per well in 6 well plates. Transwells containing CaCo-2 cells were then transferred into the multi-well plates, preloaded with J774.2 cells (Figure 2.2). CaCo-2 cells were treated with dilutions of seaweed extracts for 24 hours and J774.2 cells were stimulated with 1ug/ml LPS for 24 hours. Supernatants from apical and basolateral sides were collected for further analysis. Cultured cells were harvested and stored at -80°C in RNAlater for RNA extraction and RT-PCR.

2.6 Interleukin-8 ELISAs

Interleukin-8 Duoset ELISA's (R&D systems) were carried out as per manufacturer's instructions. Microtiter plates were coated with IL-8 capture antibody and incubated overnight at room temperature. Block buffer was added and the plate was washed three times with wash buffer. IL-8 standards and cell supernatants were added and plate was incubated for 2 hours. The plate was washed three times and detection antibody was added to each well and incubated for 2 hours. The wash step was repeated and Streptavidin-HRP was added to the plate and incubated for 20 minutes. The plate was washed three times and the substrate solution was added and incubated for 20 minutes. A stop solution was added to halt the colour reaction and optical density was read at 450nm.

2.7 RNA Extraction & RT-PCR

Total RNA was extracted from CaCo-2 cells using Roche High Pure RNA Isolation kit according to manufacturer's protocol. RNA with an A260/A280 ratio within 1.8 -2 was used for PCR. The reverse transcription of the RNA was performed using Roche Transcriptor First Strand cDNA Synthesis Kit according to manufacturer's protocol. Quantitative PCR was carried out using Roche Lightcycler 480 Probes Master kit according to manufacturer's instructions. After an initial incubation at 94°C for 15 min, qPCR was performed with 45 cycles for the housekeeper gene GAPDH and the gene of interest, IL-8. The PCR protocol was as follows: of denaturation (95°C, 15 s), annealing (48°C, 30s), and extension (72°C, 20s). The oligonucleotide primers and dual labelled probes used are listed out in Table 2.2.

2.10 Statistics

All analysis was carried out in triplicate. Results are presented as mean value plus standard error. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test and Student's T-test. (Prism 5, GraphPad Inc., San Diego, CA, USA). A *P*-value < 0.05 was considered statistically significant. Within the range of significant values the following symbol were used *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2.1: Extract information provided by SeaRefinery partners Marinox and Cyber colloids

Extract Code	Seaweed Species*	Extraction Solvent	Origin	Harvesting Time
MX121216	*S. latissima*	Water	Norway	May-16
MX221216	*A. esculenta*	Water	Norway	May-16
MX040716	*F. vesiculosus*	Water	Norway	Dec-16
ETAUG1608	*S. latissima*	Water	Ireland	July-16
CC3702	*A. nodosum*	Methanol	Irish	Jan-16
CC3762	*A. nodosum*	Ethanol	Scottish	Mar-16
CC3764	*A. nodosum*	Ethanol	Irish	Apr-16

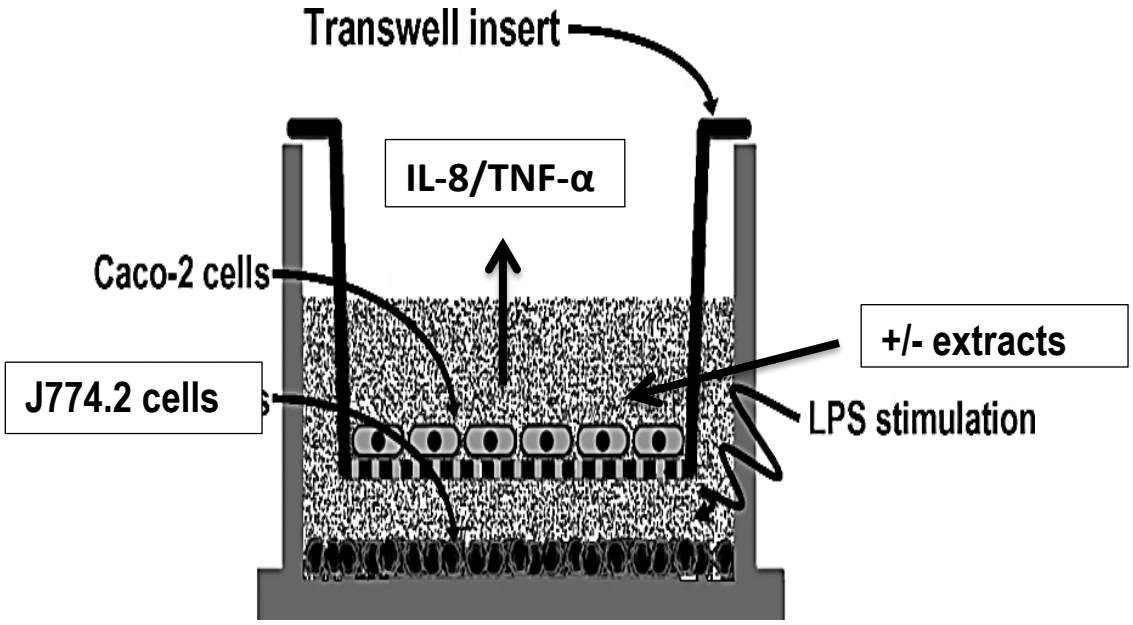


Figure 2.2: *In-vitro model of gastrointestinal inflammation. Transwell inserts on which CaCo2 cells have been cultured were inserted into multi-well plates containing J774.2 cells. To simulate gastrointestinal inflammation, lipopolysaccharide (LPS) was added to the basolateral side. Seaweed extracts were added to the apical side and IL-8 and TNF-α levels were measured after 24hrs.*

* Extracts from Saccharina latissima, Alaria esculenta, Fucus vesiculosus and Ascophyllum nodosum originated from different locations and were harvested at different time points. A variety of extraction methods were also used

Table 2.2: Forward and reverse oligonucleotide primer sequences used for qPCR.

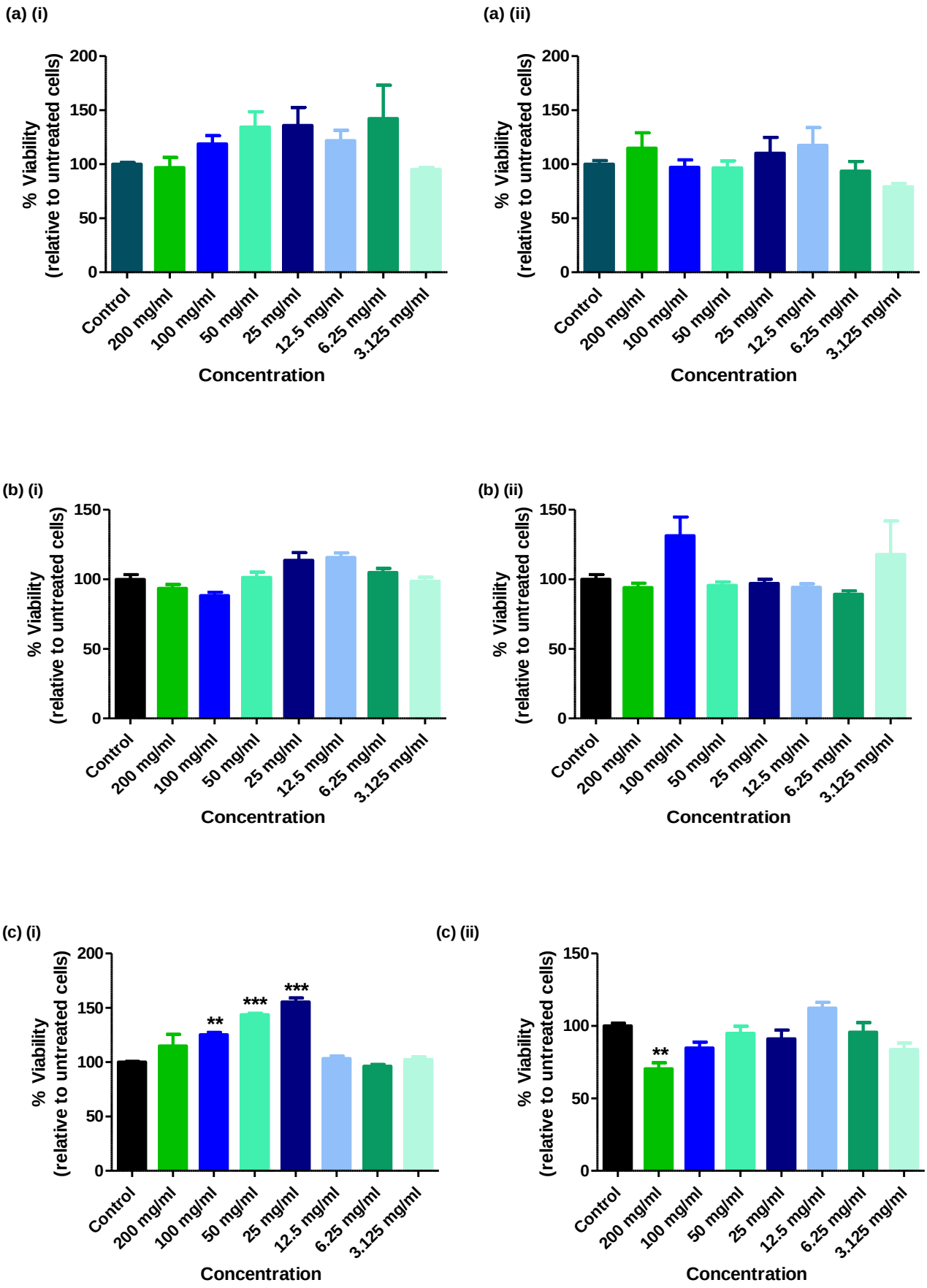
Gene	Sequence (5' – 3')
GAPDH	CTGCTCACATATTCTGGA
	CACTCACCATGTAGTTGA
Probe	[6FAM]ATGCCTTCTTGCCTCTTGTCTCTTA[BHQ1]
CXCL8	ACGAGGTGTCTATGTAAG
	GACTGATTCAGTTCACTATC
Probe*	[6FAM]ACTCACTCATACAGCATCACTAAGACA[BHQ1]

* Hydrolysis probes for each primer pair are shown

3. Results

3.1 Cytotoxicity of *Saccharina latissima, Ascophyllum nodosum, Alaria esculunta* & *Fucus vesiculosus* extracts

Potential cytotoxic effect of seaweed extracts on intestinal epithelial cell line CaCo2 and murine macrophage cell line J774.2 were assessed using Neutral Red Uptake assay. Extracts from *Saccharina latissima*, *Ascophyllum nodosum* and *Alaria esculenta* were diluted within the range of 200-3.125mg/ml (Table 2.1). No significant cytotoxic effects were observed in either cell line treated with *Saccharina* samples ETAUG1608 or MX121216, when compared with untreated cells (Figure 2.3(a), (b)). *Ascophyllum* sample CC3702 was found to significantly promote viability of CaCo2 cells at concentrations of 100mg/ml ($p<0.01$), 50mg/ml and 25mg/ml ($p<0.001$), while the higher concentration of 200mg/ml significantly reduced the viability of J774.2 cells ($p<0.01$) (Figure 2.3(c)). Higher concentrations (200mg/ml – 25mg/ml) of CC3762 and CC3764 also significantly increased viability of CaCo2 cells ($p<0.001$) (Figure 2.3 (d)(i), Figure 2.3(e)(i)). No significant change in viability was observed in J774.2 cells treated with CC3762 while cells treated with 200mg/ml ($p>0.01$), 100mg/ml and 50mg/ml ($p>0.001$) of CC3764 had significantly higher viability when compared with untreated cells (Figure 2.3(d)(ii), Figure 2.3(e)(ii)).


(a) (i)
(a) (ii)
(b) (i)
(b) (ii)
(c) (i)
(c) (ii)
% Viability
(relative to untreated cells)
Concentration
Control
200 mg/ml
100 mg/ml
50 mg/ml
25 mg/ml
12.5 mg/ml
6.25 mg/ml
3.125 mg/ml
**
***
***

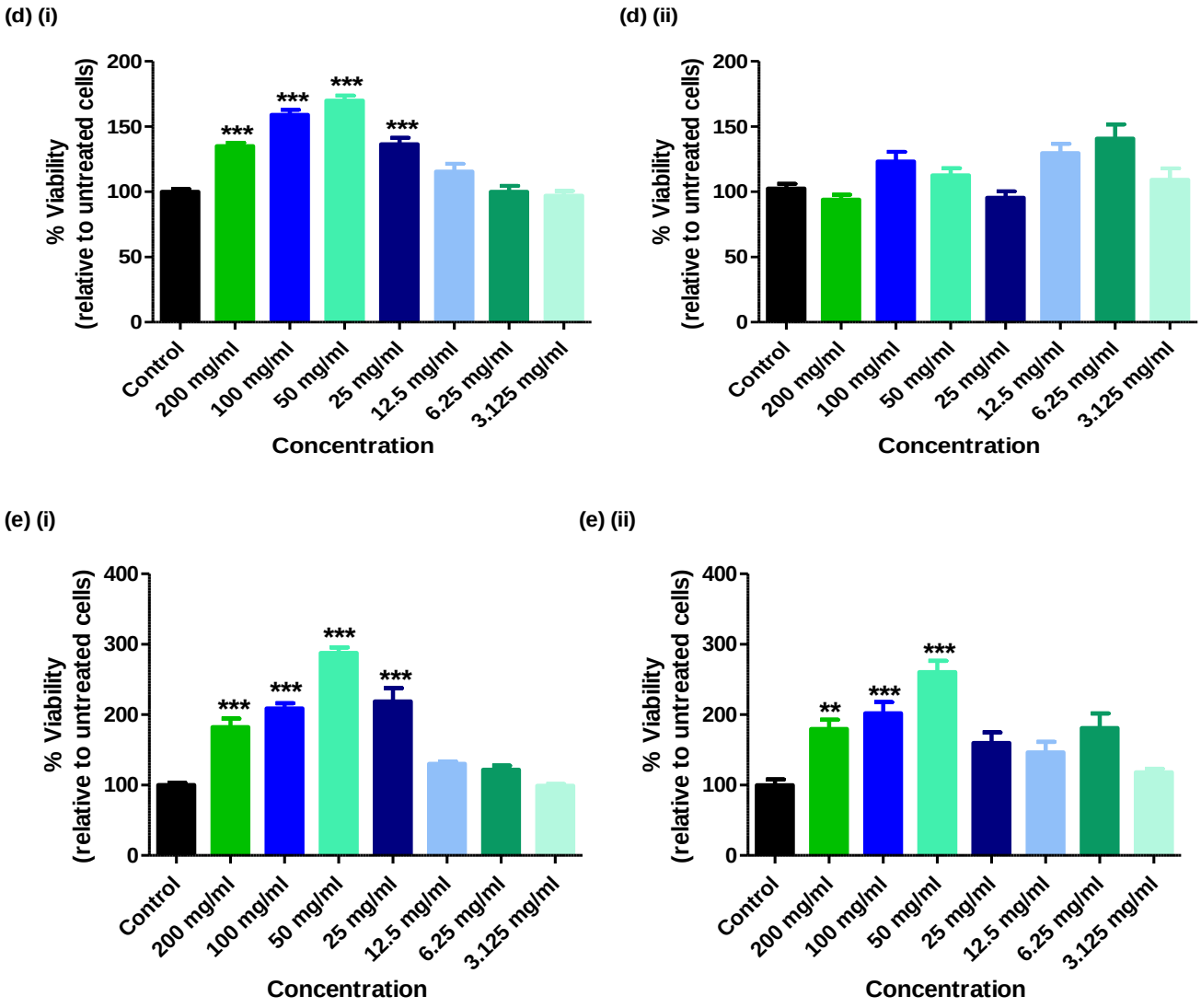


Figure 2.3(a), (b), (c), (d), (e): *Percentage viability of (i) CaCo2 cells and (ii) J774.2 cells after overnight treatment with (a) ETAUG1608, (b) MX121216 (c) CC3702, (d) CC3762 and (e) CC3764. Statistical analysis completed using one-way ANOVA where *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$*

3.2 Anti-inflammatory properties of seaweed extracts

In order to progress with testing seaweed extracts in an *in-vitro* model of gastrointestinal inflammation, anti-inflammatory properties of extracts needed to be evaluated. Expression of pro-inflammatory chemokine Interleukin-8 in the presence of extracts plus bacterial lipopolysaccharide was evaluated using ELISA assays. *Saccharina* sample MX121216, *Alaria* extract MX221216 and *Fucus* extract MX040517 from Denmark demonstrated potent inhibitory activities against the secretion of IL-8. In all three extracts, all dilutions (200mg/ml – 1.5625mg/ml) significantly inhibited IL-8 production ($p < 0.001$) (Figure 2.4(a), (b), (c)). When compared to the positive control (i.e. LPS treated cells), percentage inhibition of IL-8 production by cells treated with median concentrations of extracts (25mg/ml) were 64.4%, 73.1% and 66.1% for MX121216, MX221216 and MX040517, respectively. *Ascophyllum* extract CC3764 had a similar broad inhibitory effect on IL-8 production. CC3764 significantly inhibited IL-8 production at a concentration range of 200mg/ml – 3.125mg/ml ($p < 0.001$) with median concentration of 25mg/ml inhibiting IL-8 production by 86.8% when compared with the positive control (Figure 2.4(d)).

Ascophyllum extracts CC3702 and CC3762 and *Saccharina* extract ETAUG1608 demonstrated a narrower inhibitory range against IL-8 production. CC3702 and CC3762 inhibited Il-8 protein expression at concentrations ranging from 200mg/ml – 12.5mg/ml ($p < 0.001$). While inhibitory effects of ETAUG1608 were observed at concentrations range of 200mg/ml – 25mg/ml (Figure 2.4(e), (f), (g)). When compared to LPS treated cells, IL-8 levels in cells treated with 25mg/ml extracts were inhibited by 90.1%, 86.9% and 65.3% for CC3702, CC3762 and ETAUG1608, respectively.

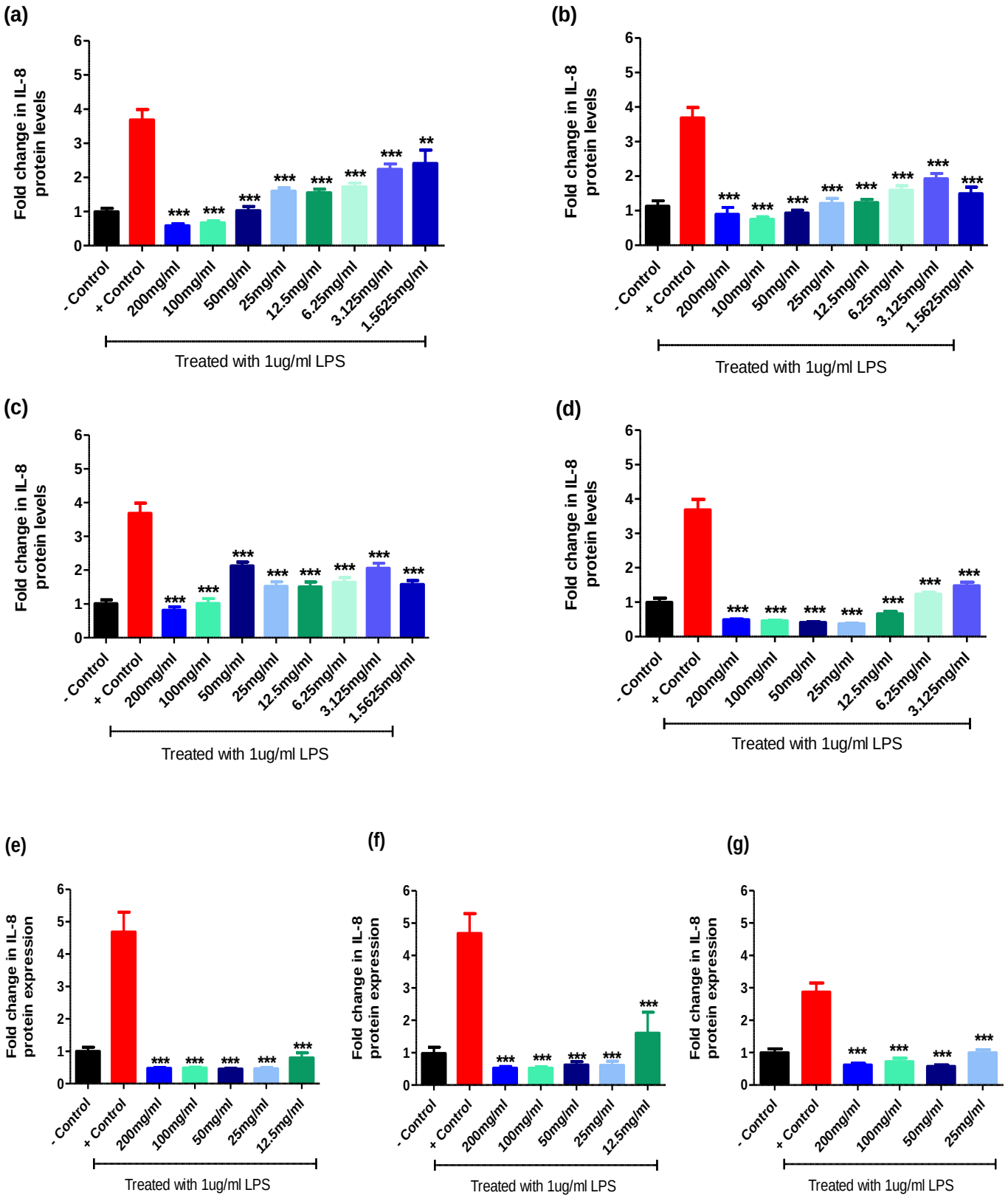


Figure 2.4 (a), (b), (c), (d), (e), (f), (g) : *Fold change in IL-8 protein expression after 24hr treatment with LPS plus concentrations of (a) MX121216, (b) MX221216, (c) MX040517, (d) CC3764, (e) CC3702, (f) CC3762 and (g) ETAUG1608. Statistical analysis completed using one-way ANOVA where *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$*

3.3 *In-vitro* model of gastrointestinal inflammation

Once the anti-inflammatory activities of the extracts were demonstrated and a viable concentration chosen (25mg/ml), extracts were tested in an *in-vitro* model of gastrointestinal inflammation (Figure 2.2). This concentration was chosen as it was the lower concentrations (12.5mg/ml -3.125mg/ml) demonstrated decreased inhibitory activities against IL-8 production. This model, composed of human intestinal epithelial cells and murine immune cells, is used to simulate gastrointestinal inflammation as it occurs in the host. All extracts at 25mg/ml significantly reduced IL-8 levels in the in-vitro model ($p<0.001$) (Figure 2.5(a)). *Ascophyllum* extracts CC3702, CC3762 and CC3764 demonstrated similar levels inhibition of IL-8 (88.2%, 87.8% and 88.5%, respectively) while in cells treated with *Alaria* extract MX221216 and *Fucus* extract MX040517 had 80.5% and 59.5% inhibition, respectively, of IL-8 respectively when compared with the positive control. In contrast to the *Ascophyllum* samples, the two *Saccharina* samples ETAUG1608 and MX121216 displayed differing levels of IL-8 inhibition. ETAUG1608 inhibited IL-8 release by 67.3%, while MX121216 demonstrated a 79.1% inhibition of IL-8. Potential inhibitory activities of chosen seaweed extracts against mRNA expression of IL-8 were also investigated. All extracts displayed significant suppressive effects against IL-8 mRNA expression (Figure 2.5(b)) ($p<0.05$). *Saccharina* extracts ETAUG1608, *Fucus* extract MX040517 and *Alaria* extract MX221216 ($p<0.001$) had more significant inhibitory activities against IL-8 mRNA expression when compared with the other extracts. Between the *Ascophyllum* extracts, CC3764 had a more significant inhibitory action ($p<0.01$) against IL-8 mRNA expression, when compared with CC3702 and CC3762 ($p<0.05$).

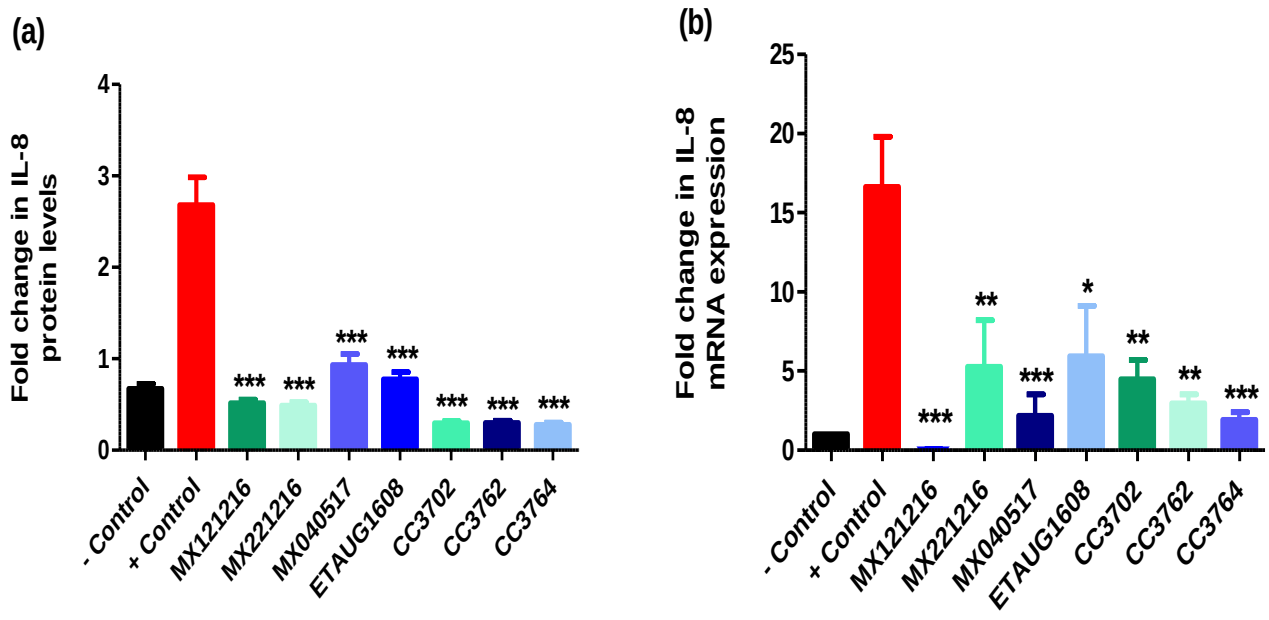


Figure 2.5 (a), (b): *Fold change in IL-8 protein levels and mRNA expression after 24hr treatment with seaweed extracts in an in-vitro model of gastrointestinal inflammation. Statistical analysis completed using one-way ANOVA where *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$*

Table 2.3: Fold change in IL-8 protein and mRNA expression after treatment with seaweed extracts.

Extract	Seaweed species	Fold change in IL-8 mRNA expression*	Fold change in IL-8 protein expression
+ Control	*N/A*	16.63	2.68
MX121216	*S. latissima*	0.05	0.52
MX221216	*A. esculunta*	5.27	0.49
MX040517	*F. vesiculosus*	2.18	0.93
ETAUG1608	*S. latissima*	5.94	0.77
CC3702	*A. nodosum*	4.48	0.30
CC3762	*A. nodosum*	2.97	0.30
CC3764	*A. nodosum*	1.94	0.28

* Results calculated based off negative control. All values expressed as average of triplicate experiments

4. Discussion

Inflammatory bowel disease (IBD) is a chronic inflammatory condition which affects the gastrointestinal tract and is often linked with increased risk of developing colorectal cancer, due to the role of chronic inflammation in the development of gastrointestinal malignancies. Current therapeutic methods to manage IBD include the use of non-biological agents and Anti-TNF agents, which manage symptoms of the disorder and can induce remission through the suppression of the immune system. However, while these treatments are generally regarded as safe, not all patients respond to these methods and side effects such as nausea, abdominal pain, development of opportunistic infections and development of malignancies are a concern (Rogler, 2010; Stallmach *et al*., 2010). As a result, research has turned to natural bioactives with minimal side effects as a means to manage IBD. Seaweed extracts have recently become of interest due to their potent bioactive properties, including immunomodulatory and anti-inflammatory activities, which could be utilised in the management of inflammatory conditions. The main objective of the present study was to investigate the anti-inflammatory properties of extracts from four species of brown seaweed i.e. *Saccharina latissima*, *Ascophyllum nodosum*, *Alaria esculenta* and *Fucus vesiculosus*. Brown macroalgae used in the study were harvested from different locations, at different time-points and extracts were isolated using water, methanol and ethanol extraction methods.

In order to determine suitability of extracts, potential cytotoxic effects were first investigated. While the *Saccharina* extracts ETAUG1608 and MX121216 demonstrated no cytotoxic effects, higher concentrations of *A. nodosum* extracts CC3702, CC3762 and CC3764 promoted the proliferation of the intestinal epithelial cell line, CaCo-2 as viability of treatment cells had a higher percentage viability when compared with control cells. Jiang et al. (2010) observed a similar growth promoting effect in the canine kidney cell line, MDCK when treated with concentrations of ascophyllan (Jiang *et al*., 2010). Ascophyllan is a sulphated polysaccharide which is distinguishable from fucoidan by its bioactivities and by the presence of a backbone of uronic acid with fucose-containing branches (3-O-D-xylosyl-l-fucose-4-sulfate). As fucoidan from the same *A. nodosum* proved cytotoxic to MDCK cells and ascophyllan has not been found in *Saccharina* species, it implies that the proliferative effect of the crude *A. nodosum* extracts observed in this study may be attributed to their ascophyllan content (Jiang *et al*., 2010). One of the main features which characterize IBD is severe damage to the epithelial layer by the inflammatory environment. Though current immunomodulatory

therapies for IBD, including anti-TNF agents, demonstrate good control of inflammatory responses, regeneration of the epithelial layer is regarded as poor (Okamoto, 2011). It has been suggested that long term remission of IBD is linked to 'mucosal healing', which can be defined as the complete repair of the epithelial layer at both endoscopic and microscopic level (Pineton de Chambrun *et al*., 2016). As such, mucosal healing is a therapeutic target for the management of IBD. Current treatments used for IBD have been found to be capable of promoting mucosal healing, though effectiveness is difficult to assess due to different study designs, different definitions of mucosal healing and different timing of endoscopic examinations (Papi *et al*., 2013). Therefore, when investigating natural alternatives treatments of IBD, bioactives with both anti-inflammatory properties and ability to induce mucosal healing are ideal. The proliferative effect of *Ascophyllum* extracts observed in this study could be utilised in the promotion of mucosal healing in IBD, though further study is required.

Abnormal inflammatory responses such as the over-production of pro-inflammatory cytokines are a common feature in the pathophysiology of IBD. Bioactives isolated from seaweed such as fucoxanthin, fucoidan, polyphenols and algal lipids have been shown to have potent immunomodulatory and anti-inflammatory activities against a number of inflammatory mediators and pro-inflammatory cytokines which could be utilised in the treatment of IBD (Kim *et al*., 2010; Heo *et al*., 2012; Park *et al*., 2011; Wijesekara *et al*., 2011; Robertson *et al*., 2015). In order to assess suitability of brown seaweed extracts in the treatment of IBD, this study investigated potential anti-inflammatory properties of the extracts against interleukin-8 production. Interleukin-8 (IL-8) is a chemotactic cytokine which is produced in high concentration in patients with IBD and other forms of colitis (McCormack *et al*., 2001). Induction of IL-8 in the intestinal epithelium triggers recruitment of neutrophils to the lamia propria, though activation, mucosal injury and trans-epithelial migration requires additional activation signals (Kucharzik *et al*., 2005). All extracts tested in this study significantly inhibited IL-8 levels in CaCo-2 cells stimulated with lipopolysaccharide (LPS), an inflammatory bacterial component. However, four extracts were found to have a wider range of inhibitory activities against IL-8 levels when compared with the other extracts. The lowest inhibitory concentration observed in *S. latissima* samples were 25mg/ml, for the Irish *S. latissima* sample ETAUG1608, and 1.5625mg/ml, for the Norwegian *S. latissima* samples MX121216. As all *S. latissima* samples were extracted using a water extraction method and a similarly wide inhibitory range was observed in the Norwegian

A. esculenta and *F. vesiculosus* extracts, this implies that origin of the source seaweed has an impact on the bioactivity of the extracts. Seaweed origin also had an impact on *A. nodosum* extract efficacy as Scottish *A. nodosum* CC3762 had a narrower inhibition range than Irish *A. nodosum* CC3764, with the lowest inhibitory concentration identified as 12.5mg/ml while the lowest inhibitory concentration of CC3764 was identified as 3.125mg/ml. However, the origin of source seaweed may not be the only factor impacting bioactivity of extracts. For example, harvesting season has been identified in several studies as a source of variation which is due to the changes in water temperature, light intensity, salinity and presence of essential nutrients caused by the changing seasons. Dar *et al*., (2007) found that extracts from the tropical seaweed *Sargassum wightii* harvested in spring (February – April) and summer months (May – July) displayed weaker anti-inflammatory properties when compared to those harvested in winter months (November – January). This differs from results obtained in this study for the *A. nodosum*, as extract harvested in winter and spring months (CC3702 and CC3762 respectively) has a narrower inhibitory range against IL-8 release when compared with the extract harvested in summer (CC3764). Therefore, in order to optimize bioactive efficacy, optimum harvesting season for each seaweed species should be determined.

The anti-inflammatory activities of these brown seaweed extracts were further demonstrated using an *in-vitro* model of gastrointestinal inflammation, as established by Tanoue *et al*., (2008). The *in-vitro* model, comprised of human intestinal epithelial cells and murine immune cells, was established as a means to determine anti-inflammatory activities of food factors at the cellular level. Murine immune cells are stimulated with bacterial lipopolysaccharide, which then affects the layer of epithelial cells, thereby simulating the abnormal immune responses observed in IBD and other forms of colitis. All seaweed extracts tested at the chosen concentration of 25mg/ml significantly reduced IL-8 protein levels in this *in-vitro* model. This concentration was chosen as it was the lowest effective inhibitory concentration. Lower concentrations demonstrated decreased inhibitory activities against IL-8 production. However, the *A. nodosum* and *A. esculenta* extracts demonstrated a higher IL-8 inhibition (>80%) when compared with the *F. vesiculosus* extract (59.5%) indicating that this species of seaweed may have weaker anti-inflammatory properties. The Norwegian *S.latissima* demonstrated a higher degree of IL-8 inhibition (79.1%) when compared with the Irish S.latissima (59.5%), which could be attributed to differences in seaweed origin and seasonal change. The

anti-inflammatory properties of these extracts could be attributed to their suppressive effects on IL-8 mRNA expression. Similar suppressive effect of seaweed extracts on protein levels and mRNA expression of pro-inflammatory cytokines has been observed in other studies (Wijesinghe *et al*., 2013; Park *et al*., 2011).

It is clear that extracts from brown seaweed species, particularly from *Saccharina latissima*, *Alaria esculenta,* and *Ascophyllum nodosum*, have potent anti-inflammatory properties which could be utilised in the treatment of gastrointestinal inflammatory disorders. The anti-inflammatory properties of these extract have been observed in normal physiological conditions, indicating that the use of these seaweed extracts as anti-inflammatory agents should only be prescribed to those in diseased states (Appendix I). However, influence of seasonal change and origin of harvested material should be considered as barriers to the year round formulation of consistent bioactives. Further assessment of the these extracts could include determining potential inhibitory effects on additional pro-inflammatory chemokines and cytokines, including MCP-1, CCL20, IL-6 and IL-1β, and assessing potential suppressive effect on inflammatory mediators such as nitric oxide production, prostaglandin E_2 (PGE_2) production and nuclear factor-kappa (NF-κB) activation. Extracts should also be further assessed using animal models and human trials in order to determine whether these extracts have similar effects *in-vivo* as *in-vitro.*

Chapter 3

Anti-microbial properties of brown seaweed extracts

1. Introduction

Seaweeds are classified as Rhodophyta (red macroalgae), Chlorophyta (green macroalgae) or Phaeophyta (brown macroalgae) depending on their pigmentation, structure and biochemical composition. These marine plants survive and live in complex environments and are often exposed to varying environmental conditions. In response to these conditions, marine macroalgae (particularly those in fixed positions) produce a number of secondary metabolites which may aid in survival. A number of these metabolites limit the growth of competitive microorganisms and prevents the settlement of fouling organisms (Zerrifi *et al*. 2018). Algal extracts and their purified components have also been found to exhibit anti-oxidant, anti-cancer, anti-inflammatory and anti-coagulant activities which are of interest to the food and pharmaceutical industry (O'Sullivan *et al*. 2011; Kim *et al*. 2010; Robertson *et al*. 2015; Cumashi *et al*. 2007).

Brown seaweed extracts have been reported to be active against a number of Gram negative and Gram positive bacteria, with a greater efficacy than extracts from red and green seaweed (Gupta *et al*., 2010; Gupta *et al*., 2012; Cox., 2010). This may be due to a selection of bioactive compounds found in brown seaweed which are not be found in red or green seaweed including fucoidan, a sulphated polysaccharide mainly found in the cell wall of brown macroalgae, phlorotannin, a tannin only found in brown macroalgae, and fucoxanthin, a pigment which gives brown macroalgae its colour. These bioactives have shown inhibitory activities against a number of microorganisms (Liu *et al*., 2017; Vijayabaskar *et al*., 2012; Eom *et al*., 2012; Rajauria & Abu-Ghannam, 2013). Some studies have attributed antimicrobial activity with phenolic content of brown seaweed extracts, as phenolic compounds have several modes of actions which could inhibit bacterial growth, such as damaging the microorganism's cell walls and cell membranes resulting in the release of intracellular components (Gupta & Abu-Ghannam, 2011).

An area in which the anti-bacterial properties of seaweed extracts could be utilised is in the maintenance of gastrointestinal health. The gastrointestinal tract hosts a complex community of symbiotic micro-organisms, which interacts with the digestive tract, to promote gut homeostasis. This gut microbiota consists of approximately 100 trillion micro-organisms, including bacteria, fungi and viruses, in varying concentrations, along the GI tract. The gut microbiota plays a vital role in maintaining host health as it is involved in the development of healthy immune responses, acts as a natural defensive

barrier against infection and is involved in metabolic processes such as the anaerobic fermentation of carbohydrates and the proteolytic fermentation of metabolites such as phenolic compounds, amines and ammonia. As the gut microbiota is intrinsic to host health, imbalances in the microbial community caused by environmental changes such as diet and lifestyle have been linked to the pathology of certain gastrointestinal disorders such as obesity, colorectal cancer and inflammatory bowel disorder (IBD). For example, microbial dysbiosis is a common symptom associated with IBD and is thought to contribute to the chronic inflammatory responses observed in this disorder. Common treatments of IBD also involve the use of immune supressing agents, which may promote susceptibility to hospital or foodborne infections.

The aim of this chapter was to determine potential anti-microbial properties of extracts from three brown seaweed species : *Saccharina latissima*, *Alaria esculenta* and *Ascophyllum nodosum* against a number of bacteria relevant to gastrointestinal health (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Salmonella enterica*). These bacterial species were selected due to their potential role in the pathogenesis of IBD or due to their potential role as causative agents of infection in immunocompromised patients with IBD. A probiotic strain, *Lactobacillus johnsonii*, was also tested with these extracts in order to investigate potential activity against normal flora of the host and to determine if there are any prebiotic activities of these extracts.

2. Materials and Methods

2.1 Seaweed Materials

Extracts from brown macroalgae species *Saccharina latissima*, *Ascophyllum nodosum* and *Alaria esculenta* were provided by SeaRefinery partners Cybercolloids and Marinox (Table 3.1). The brown macroalgae were harvested from different locations (Ireland, Denmark and Scotland) (Table 3.1). *Saccharina latissima* and *Alaria esculunta* extracts were isolated by means of water extraction with differing protocols (Table 3.1). *Ascophyllum nodosum* extracts from different locations and different seasons were isolated by means of methanol, water or sodium hydroxide and ascorbic acid extraction (Table 3.1). Dried extracts were dissolved in phosphate buffer saline solution (PBS).

2.2 Microbial culture

Five species of bacteria were selected based on their relevance to gastrointestinal health, the food industry and clinical background. The bacteria selected were *Staphylococcus aureus*, *Enterococcus faecalis*, *Lactobacillus johnsonii*, *Escherichia coli* and *Salmonella enterica* subsp. *typhimurium*. All cultures were maintained at -80°C in 40% glycerol and grown in Tryptic Soy Broth (TSB) at 37°C except for *Lactobacillus johnsonii* which was grown in De Mann, Rogosa and Sharpe (MRS) broth at 37°C.

2.3 Growth curves in the presences of seaweed extracts

The influence of different concentrations of seaweed extracts on the growth kinetics of the seven organisms was assessed using 96 well microtiter plates. Overnight cultures of bacteria were diluted to 0.5 McFarland standards in sterile Ringers. Suspension was then diluted 1:100 in sterile broth to achieve a final cell concentration of $1x10^6$ CFU/ml. To assess the anti-bacterial activity of seaweed, 200ul of highest concentration of seaweed extract (200mg/ml) was added to the second row of each plate. The other wells were filled with 100ul broth and 100ul from the first well was serial diluted 2-fold along each column. Equal volumes of bacterial suspension were added to each well. The first row of each plate was used for bacterium and media controls. Samples blanks for each concentration of the extracts were also prepared. Plates were incubated for 24 hours in a plate reader at 37°C. Turbidity was measured as absorbance at 600nm with 5s agitation before each OD measurement.

2.4 Bacteriostatic assay

Extract concentrations with anti-microbial properties were then determined to be bacteriostatic or bactericidal. Anti-bacterial assays were carried out as described previously in ‘’2.3. Growth curves in the presences of seaweed extracts’’. After a 24 hour incubation, control bacteria and anti-bacterial dilutions plus bacteria were transferred to 1ml sterile Ringer’s solution in Eppendorf tubes. The tubes were then centrifuged at 1,500rpm for 5 minutes or until a pellet was formed. The pellet was washed twice with sterile Ringer’s, re-suspended and diluted to a 0.5 McFarland standards in sterile Ringers. Suspensions were then diluted 1:100 in sterile TSB to achieve a final cell concentration of $1x10^6$ CFU/ml. The microtiter plates were filled with 100ul sterile TSB broth. Equal volumes of bacterial suspension were added to triplicate wells. Plates were incubated for 24 hours in a plate reader at 37°C. Turbidity was measured as absorbance at 600nm with 5s agitation before each OD measurement. Extracts were determined to be bacteriostatic if bacteria resumed growth in fresh media.

2.5 Statistics

All analysis was carried out in triplicate. Results are presented as mean plus standard error. Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test (Prism 5, GraphPad Inc., San Diego, CA, USA). A *P*-value < 0.05 was considered statistically significant. Within the range of significant values the following symbol were used *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 3.1: Extract information provided by SeaRefinery partners Marinox and Cybercolloids.

Extract Code	Seaweed Species*	Extraction Solvent	Origin	Harvesting Time
MX121216	*S. latissima*	Water	Norway	May-16
MX221216	*A. esculenta*	Water	Norway	May-16
ETAUG1608	*S. latissima*	Water	Ireland	July-16
CC3689	*A. nodosum*	Sodium hydroxide & ascorbic acid	Ireland	Jan-16

* Extracts from *Saccharina latissima*, *Alaria esculenta* and *Ascophyllum nodosum* originated from different locations and were harvested at different timepoints. A variety of extraction methods were used.

3. Results

3.1 Anti-bacterial & bacteriostatic properties of Norwegian *Saccharina latissima*

In order to determine the anti-bacterial properties of selected seaweed extracts, growth kinetics of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Salmonella enterica* were observed in the presence of concentrations of seaweed extracts. Extracts from Norwegian *Saccharina latissima* demonstrated growth inhibitory activities on all bacteria, but to differing degrees. When compared to the control (bacteria in media), after 24 hours, significant growth inhibition of *S. aureus* was observed at 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml ($P>0.001$) and 12.5mg/ml ($P<0.01$) (Figure 3.1(a)(i)). Percentage inhibition estimated for these concentrations were 94.25% for 200mg/ml, 100% for 100mg/ml, 50mg/ml and 25mg/ml and 29.23% for 12.5mg/ml (Table 3.2). Similar inhibitory activities were observed for *E. faecalis* at concentrations of 200mg/ml – 12.5mg/ml ($P<0.001$) (Figure 3.1(b)(i)) with percentage inhibition ranging from 82 – 100%. Minimum inhibitory concentration (MIC) of *S.latissima* extracts were determined to be 12.5mg/ml, for *S. aureus* and *E. faecalis*. In the case of *E. coli* and *S. enterica*, the *S.latissima* extract significantly inhibited growth at all concentrations with MIC identified as 6.25mg/ml ($P<0.001$) (Figure 3.1(c)(i), (d)(i)). Percentage inhibition of the highest concentration of the extract (200mg/ml) was determined to be 100% for both *E. coli* and *S. enterica*, with the inhibition rate decreasing with lower concentrations (Table 3.2). In order to determine whether anti-microbial activity of the *Saccharina* extract was bactericidal or bacteriostatic, bacteria were removed from extracts and re-grown in fresh media. As all bacteria resumed growth after removal of extract concentrations, Norwegian *S. latissima* extract was determined to be bacteriostatic. (Figure 3.1(a)(ii), (b)(ii) (c)(ii), d(ii)).

Table 3.2: Percentage inhibition of S. aureus, E. faecalis, E. coli and S. enterica in the presence of Norwegian Saccharina latissima

Concentration (mg/ml)	***S. aureus***	***E. faecalis***	***E. coli***	***S. enterica***
200	94.25	86.61	100	100
100	100	100	100	51.83
50	100	100	97.17	59.84
25	100	100	63.05	36.77
12.5	29.23	82.05	49.98	30.42
6.25	N/A	N/A	37.66	28.71

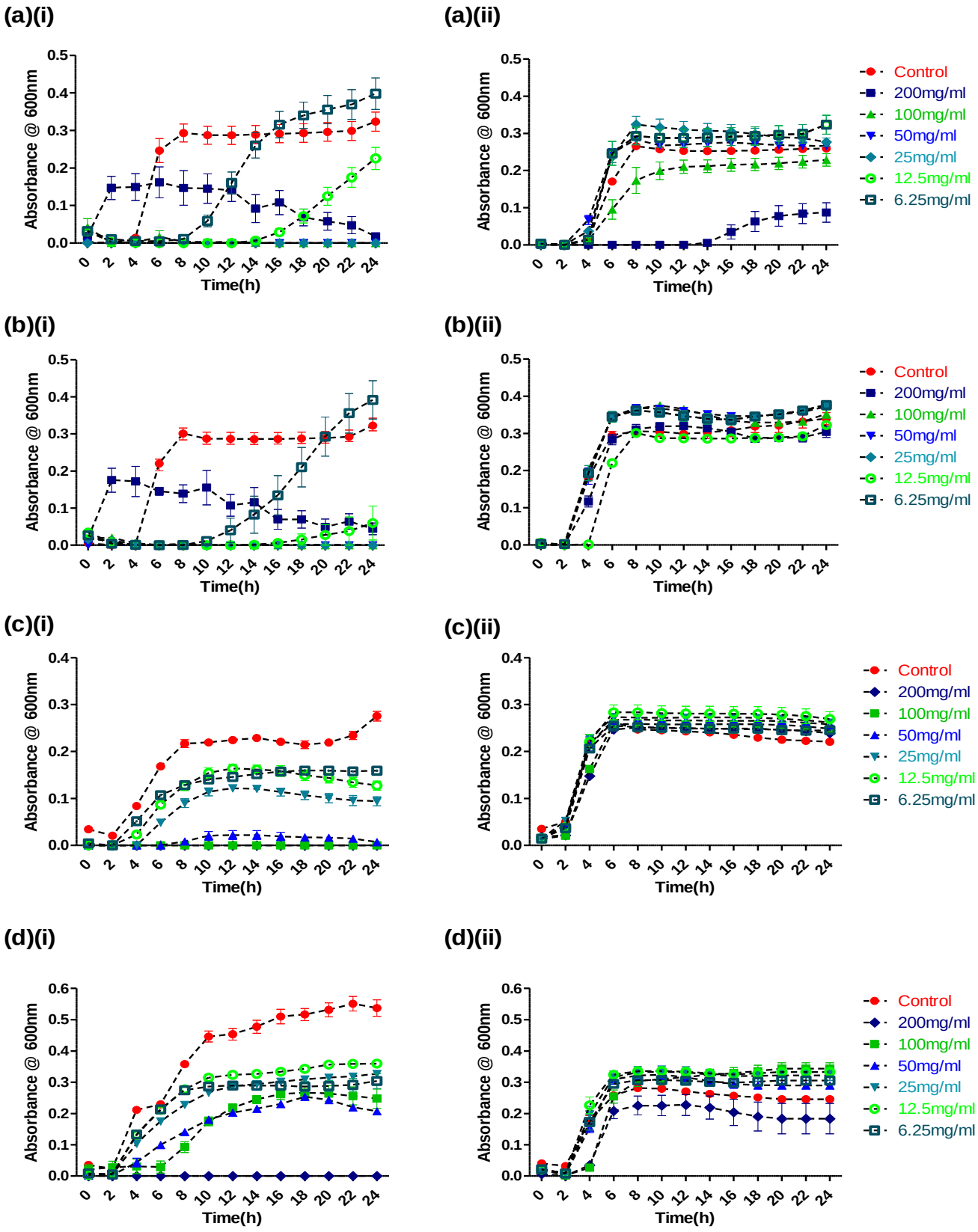


Figure 3.1: *(i) Growth kinetics and (ii) recovery of selected bacteria (a) Staphylococcus aureus, (b) Enterococcus faecalis, (c) Escherichia coli, (d) Salmonella enterica in the presence of Norwegian Saccharina latissima. Significant growth inhibition against all bacteria was observed at 200mg/ml – 12.5mg/ml when compared to control.*

3.2 Anti-bacterial & bacteriostatic properties of Irish *Saccharina latissima*

Significant growth inhibition of all bacteria was observed when grown in the presence of 200mg/ml and 100mg/ml of Irish *S.latissima* extract ($P<0.001$) (Figure 3.2(a)(i), (b)(i), (c)(i), (d)(i)). The extract demonstrated a narrow inhibition range for the chosen Gram + bacteria, *S. aureus* and *E. faecalis*, as MIC was determined to be 50mg/ml. Percentage inhibition for *S. aureus* and *E. faecalis* was estimated at 100% for 200mg/ml (Table 3.3). A wider inhibitory range was observed for the chosen Gram – bacteria, with MIC determined to be 6.25mg/ml for *E. coli* and *S. enterica* (Table 3.3). Significant growth inhibitory effects were observed at 200mg/ml – 25mg/ml for these bacteria ($P<0.001$). Percentage inhibition for *E. coli* was estimated at 100% for 200mg/ml and 88.46% for 100mg/ml. Dose dependant inhibition was observed for *S. enterica* with 100% for 200mg/ml with inhibition rate decreasing with lower concentrations (Table 3.3). As observed for the Norwegian *Saccharina* extract growth of bacteria resumed after removal of extract, indicating bacteriostatic activity rather than bactericidal (Figure 3.2(a)(ii), (b)(ii), (c)(ii), (d)(ii)).

Table 3.3: Percentage inhibition of S. aureus, E. faecalis, E. coli and S. enterica in the presence of Irish Saccharina latissima

Concentration (mg/ml)	***S. aureus***	***E. faecalis***	***E. coli***	***S. enterica***
200	100	100	100	100
100	51.61	61.24	88.46	69.99
50	0.25	6.64	29.70	73.7
25	N/A	N/A	40.64	43.0
12.5	N/A	N/A	36.60	N/A
6.25	N/A	N/A	39.92	N/A

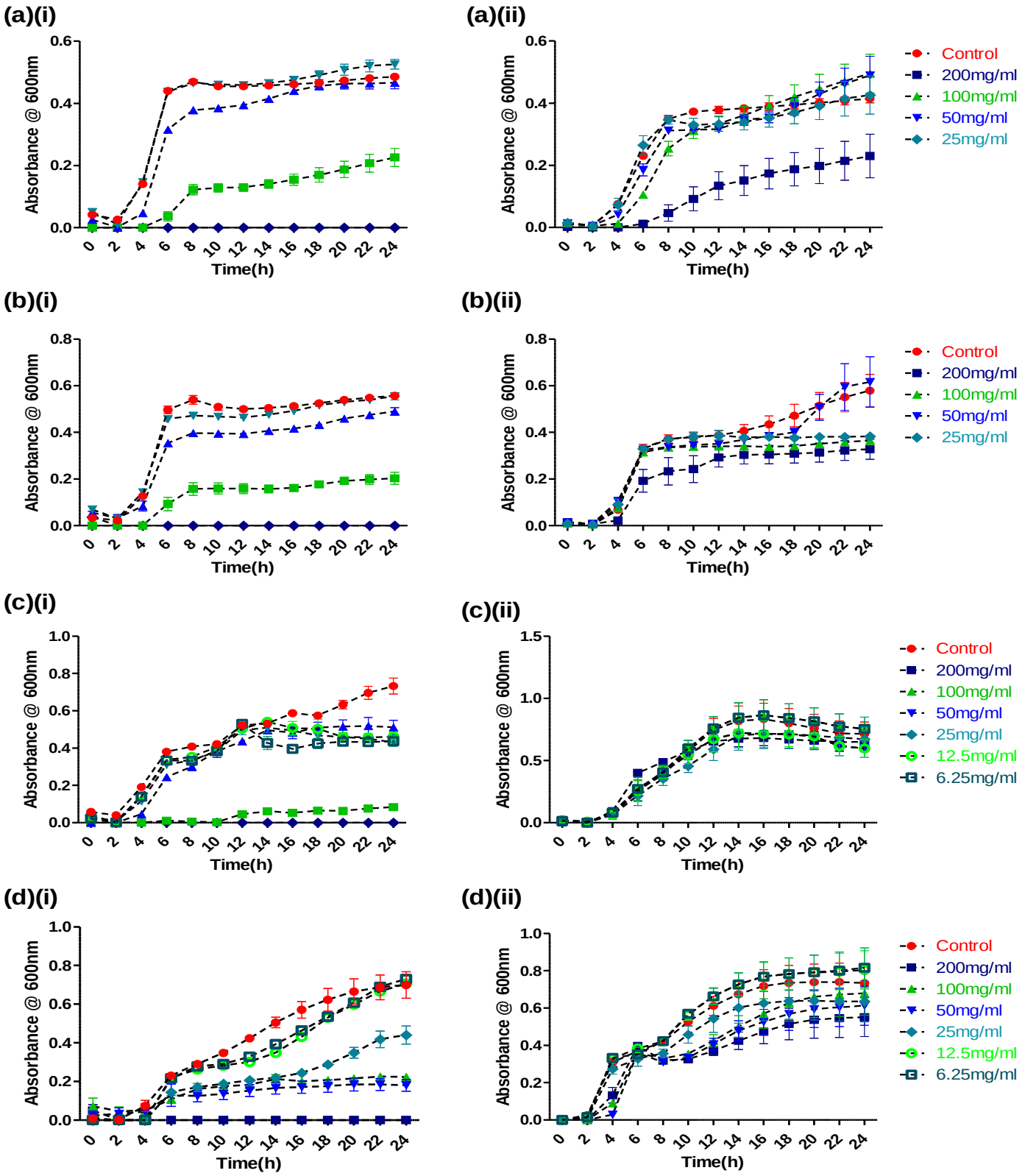


Figure 3.2: *(i) Growth kinetics and (ii) recovery of selected bacteria (a) Staphylococcus aureus, (b) Enterococcus faecalis, (c) Escherichia coli, (d) Salmonella enterica in the presence of Irish Saccharina latissima. Significant growth inhibition of S. aureus and E. faecalis were observed at 200mg/ml and 100mg/ml when compared to the control. Significant growth inhibitory activities against E. coli and S. enterica were observed at 200mg/ml – 25mg/ml.*

3.3 Anti-bacterial & bacteriostatic properties of *Alaria esculenta*

Extracts from *Alaria esculenta* demonstrated growth inhibitory activities on selected bacteria. Significant growth inhibition of *S. aureus* was observed at 200mg/ml, 100mg/ml and 50mg/ml ($P<0.001$) with percentage inhibition of these concentrations determined to be 100%, 100% and 97.82%. Similar growth inhibitory activity was observed in *E. faecalis* at 200mg/ml, 100mg/ml and 50mg/ml ($P<0.001$) (Figure 3.3(a)(i), (b)(i))(Table 3.4). MIC of the extract on tested Gram + bacteria was 25mg/ml. In the case of *E. coli*, growth was significantly inhibited at 200mg/ml ($P<0.001$) and 50mg/ml ($P>0.05$) Percentage inhibition of 200mg/ml was determined to be 100%. Growth of *S. enterica* was significantly inhibited at all concentrations ($P<0.001$) with highest concentration inhibiting growth by 70% (Figure 3.2(c)(i), (d)(i)). All bacteria resumed growth after removal of extracts, indicating bacteriostatic activity of *Alaria esculenta* (Figure 3.3(a)(ii), (b)(ii), (c)(ii), (d)(ii))

Table 3.4: Percentage inhibition of S.aureus, E.faecalis, E.coli and S.enterica in the presence of Alaria esculenta

Concentration (mg/ml)	***S. aureus***	***E. faecalis***	***E. coli***	***S. enterica***
200	100	100	100	70
100	100	100	38.02	54.83
50	97.82	84.66	51.53	46.64
25	9.29	9.67	17.73	36.46
12.5	N/A	N/A	N/A	36.54
6.25	N/A	N/A	21.89	38.37

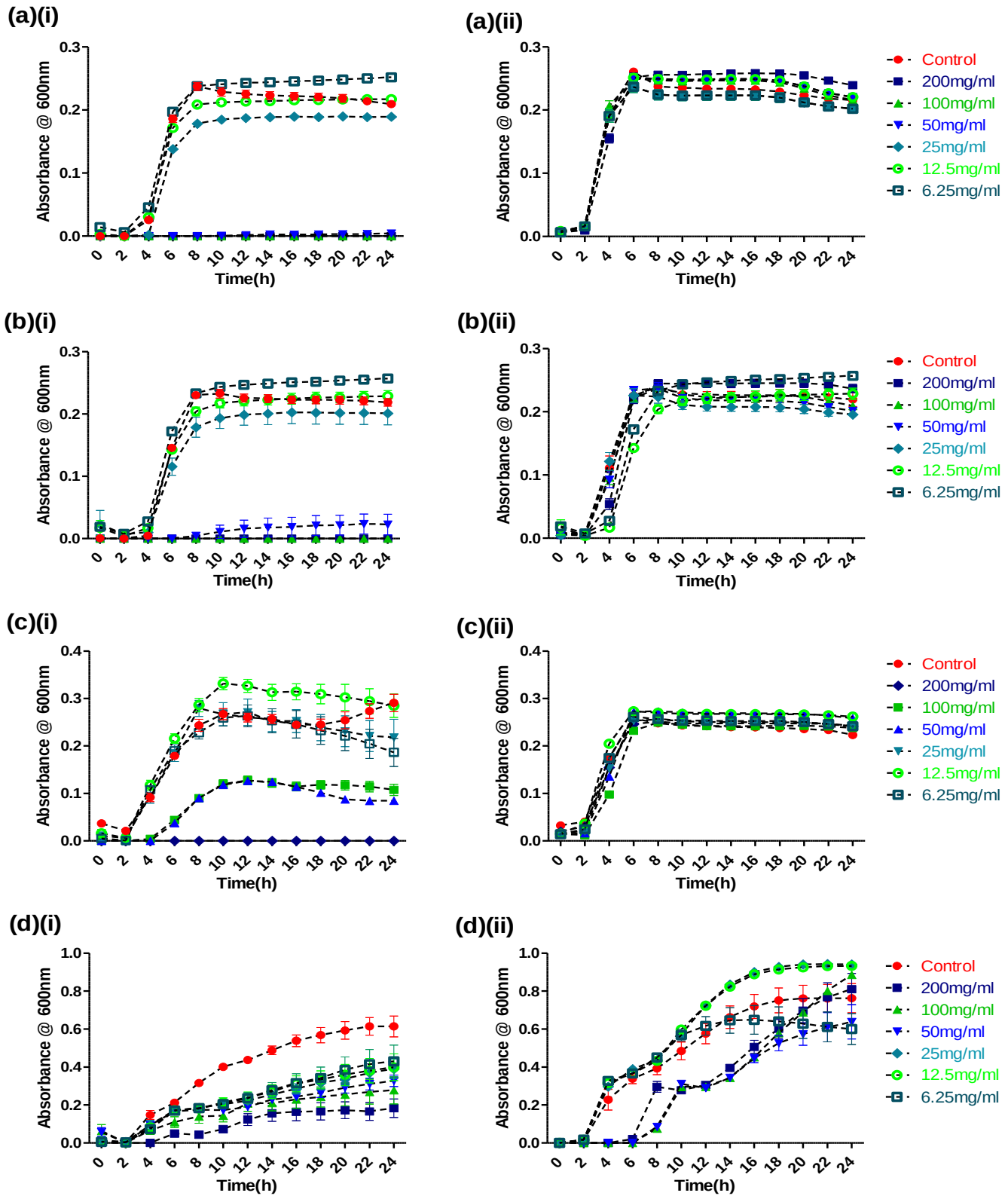


Figure 3.3: *(i) Growth kinetics and (ii) recovery of selected bacteria (a) Staphylococcus aureus, (b) Enterococcus faecalis, (c) Escherichia coli, (d) Salmonella enterica in the presence of Alaria esculenta. Significant growth inhibition of S. aureus, E. faecalis and E. coli were observed at 200mg/ml – 50mg/ml. Growth inhibition of S. enterica was observed at all concentrations of the extract when compared to the control*

3.4 Anti-bacterial & bacteriostatic properties of *Ascophyllum nodosum*

Extracts from *Ascophyllum nodosum* significantly hindered growth of chosen Gram + bacteria at concentrations of 200mg/ml, 100mg/ml and 50mg/ml (Figure 3.4 (a)(i), (b)(i)). Highest percentage inhibition of 100% was observed at 200mg/ml and MIC was determined to be 6.25mg/ml (Table 3.5). Anti-microbial effects of this extract were less effective against chosen Gram – bacteria (Figure 3.4(c)(i), (d)(i)). Significant inhibitory activities against *E. coli* were observed at 200mg/ml, 100mg/ml and 50mg/ml ($P<0.001$), ($P<0.01$) to a lesser degree than observed on *S. aureus* and *E. faecalis* as growth was inhibited by 71.51% at 200mg/ml. Significant reduction of *S. enterica* by *A. nodosum* extract was only observed at the highest concentration 200mg/ml ($P<0.001$) with a 76.39% reduction in growth. All other concentrations inhibited growth by approximately 20% with MIC determined as 25mg/ml. Growth of bacteria resumed after removal of extracts, indicating bacteriostatic activity of *A. nodosum* (Figure 3.4 (a)(ii), (b)(ii), (c)(ii), (d)(ii)).

Table 3.5: Percentage inhibition of S. aureus, E. faecalis, E. coli and S. enterica in the presence of Ascophyllum nodosum

Concentration (mg/ml)	***S. aureus***	***E. faecalis***	***E. coli***	***S. enterica***
200	100	100	71.51	76.39
100	86.75	82.21	30.45	21.31
50	50.78	78.30	11.71	19.69
25	37.01	33.93	N/A	19.7
12.5	36.97	33.33	N/A	N/A
6.25	14.38	26	N/A	N/A

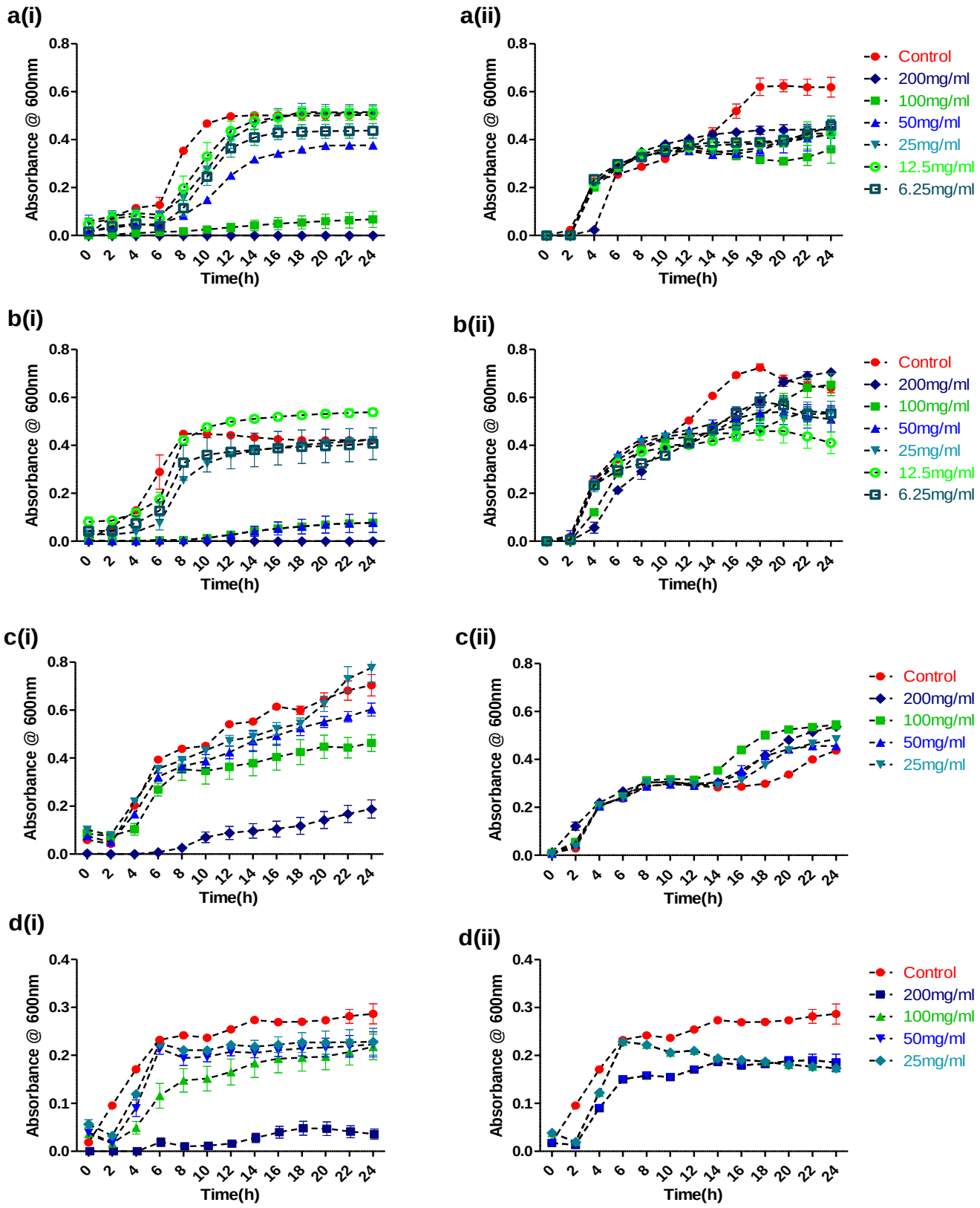


Figure 3.4: *(i) Growth kinetics and (ii) recovery of selected bacteria (a) Staphylococcus aureus, (b) Enterococcus faecalis, (c) Escherichia coli and (d) Salmonella enterica in the presence of Ascophyllum nodosum. Significant growth inhibition of S. aureus, E. faecalis and E. coli were observed at 200mg/ml – 50mg/ml when compared to the control. Growth inhibition of S. enterica was only observed at 200mg/ml.*

3.5 Growth kinetics of *Lactobacillus johnsonii* in the presence of *Saccharina latissima, Alaria esculenta* and *Ascophyllum nodosum*

In order to assess potential detrimental effects of seaweed extracts on normal human gut flora, anti-microbial activity of the extracts were assessed against probiotic strain *Lactobacillus johnsonii*. Concentrations of 200mg/ml ($P<0.001$) and 100mg/ml ($P<0.01$) of the Norwegian *S. latissima* (MX121216) were found to significantly reduce the growth of *L. johnsonii*, with a 93.78% reduction observed at 200mg/ml (Figure 3.5(a))(Table 3.6). Similar growth inhibitory effects were observed with the *A. esculenta* extract with significant growth inhibition at 200mg/ml ($P<0.001$), 100mg/ml ($P<0.01$) and 50mg/ml ($P<0.001$) (Figure 3.5(b)). However, 200mg/ml of this extract only inhibited growth by 31.38% (Table 3.6). In contrast, only the highest concentration of the Irish *S. latissima* extract (200mg/ml) caused significant growth inhibition ($P<0.001$). However, at 50mg/ml ($P<0.01$) and 25mg/ml ($P<0.001$) Irish extract promoted growth of *L. johnsonii* when compared to the control. No growth inhibition was observed in *L. johnsonii* in the presence of the *A. nodosum* extract. Concentrations of 100mg/ml, 50mg/ml and 25mg/ml promoted the growth of *L. johnsonii* when compared to the control, indicating potential prebiotic effects of *A. nodosum*. Bacterial growth resumed after removal of extracts indicating bacteriostatic activity of seaweed extracts (data not shown) (Appendix II).

Table 3.6: Percentage inhibition of Lactobacillus johnsonii in the presence of seaweed extracts*

Concentration (mg/ml)	MX121216	MX221216	ETAUG1608	CC3689
200	93.78	31.38	100	N/A
100	32.72	26.62	N/A	N/A
50	N/A	42.21	N/A	N/A
25	N/A	17.93	N/A	N/A

* Norwegian *Saccharina latissima* (MX121216), *Alaria esculenta* (MX221216), Irish *Saccharina latissima* (ETAUG1608) and *Ascophyllum nodosum* (CC3689)

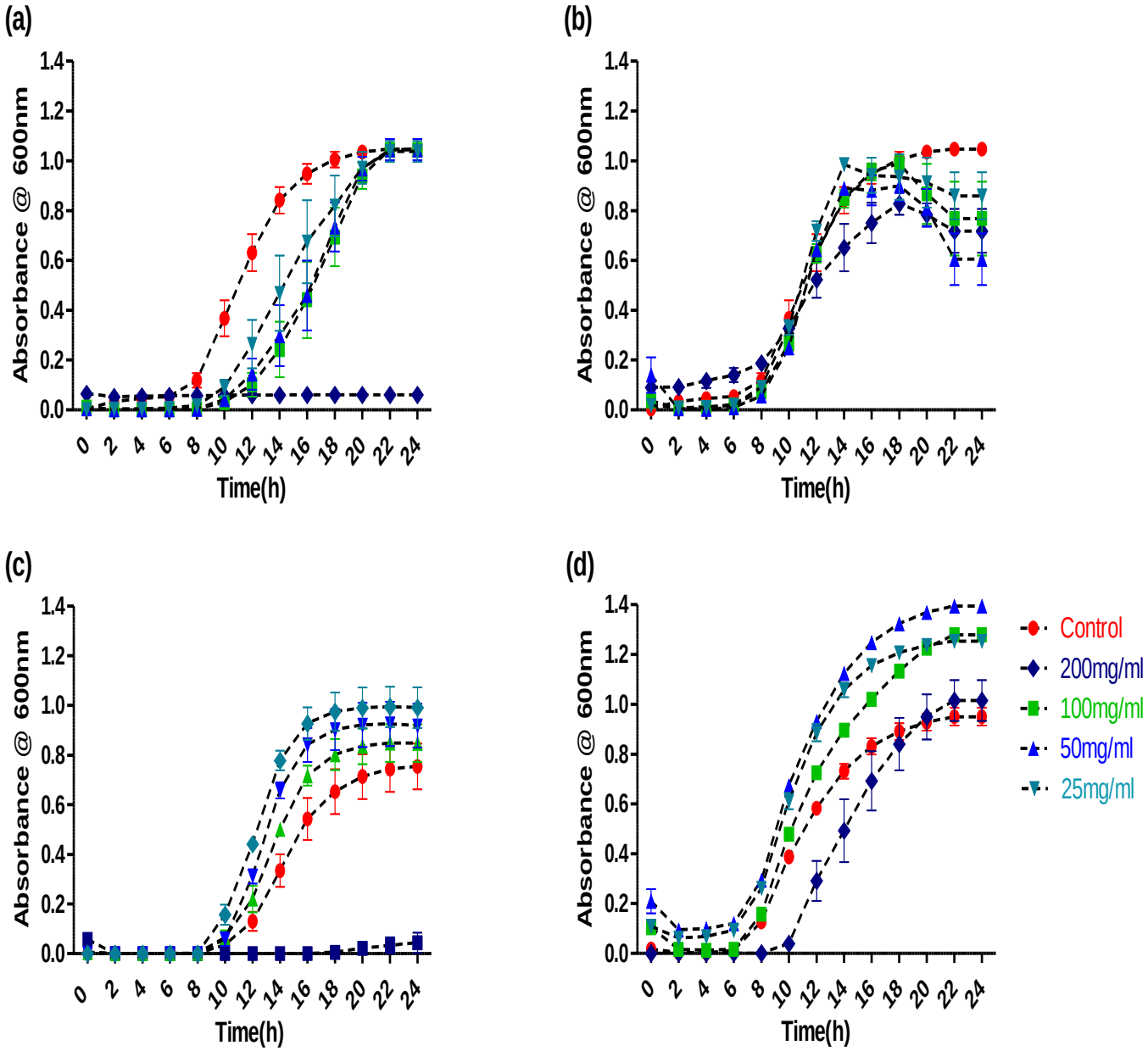


Figure 3.5: *Growth kinetics of Lactobacillus johnsonii in the presence of (a) Norwegian Saccharina latissima (MX121216) (b) Alaria esculenta (MX221216) (c) Irish Saccharina latissima (ETAUG1608) and (d) Ascophyllum nodosum (CC3689). Growth of L. johnsonii was inhibited in the presence of Norwegian S. latissima and A. esculenta. Growth of L. johnsonii was promoted in the presence of 100-25mg/ml of the Irish S. latissima and A. nodosum extracts.*

4. Discussion

Marine plants such as seaweeds live in complex communities and are often exposed to a number of environmental conditions throughout the year. Seaweeds grow in close proximity to other marine organisms and have the ability to survive with a competitive and hostile environment. Although these plants are sessile and have no physical defences, they produce a series of complex secondary metabolites in response to these ecological pressures in order to ensure survival of the seaweed. These bioactives have been linked to a number of properties which are of interest to the food and pharmaceutical industry such as anti-oxidant, anti-inflammatory, anti-cancer, anti-lipidemic and anti-diabetic (Yin Chia *et al*., 2015; Heo *et al*., 2012; Kim *et al*., 2010; Jung *et al*., 2014; Selvaraj & Palanisamy, 2014). Seaweeds have also been identified as novel sources of anti-microbial compounds (Pérez *et al*., 2016). In general, seaweeds have a symbiotic relationship with the microbial community in its natural environment, as cultivation in axenic conditions have been linked to stunted growth and abnormal morphology (Singh *et al*., 2011). However, these marine plants have been seen to produce a number of anti-microbial compounds to prevent the growth and settlement of competitive and fouling organisms (Zerrifi *et al*., 2018). Several studies have illustrated the anti-microbial properties of seaweed extracts against a number of Gram + and Gram – bacteria which could be utilised by the food and pharmaceutical industry. Brown seaweed extracts in particular have been found to have a greater efficacy when compared with red and green seaweed extracts (Cox, 2010; Gupta *et al*., 2012).

An area in which the anti-microbial properties of seaweed extracts could be of use is in the maintenance of gastrointestinal health. The gastrointestinal tract contains a complex community of microorganisms, called the gut microbiota, which plays a critical role in host health and defence. Imbalances in the gut microbiota caused by environmental conditions such as diet or by enteric infections have negative impacts on host health. In fact, the pathophysiology of several gastrointestinal disorders such as colorectal cancer and inflammatory bowel disorder (IBD) has been linked to imbalances in the microbial community. For example it has been well understood that infections can initiate onset and relapse of IBD (Irving & Gibson, 2008). Due to the nature of the disease, patients are often predisposed to infections and the use of immune suppressors as treatment methods for IBD may cause increased susceptibility to opportunistic pathogens. Patients with IBD, particularly those with ulcerative colitis, were found to have a higher incidence of *Clostridium difficile* associated diarrhoea (CDAD) when compared with

non-IBD patients, with the majority of infections acquired before hospitalization (Rodemann *et al*., 2007). The purpose of this study is to investigate the anti-microbial properties of extracts from three brown seaweed species, *Saccharina latissima*, *Alaria esculenta* and *Ascophyllum nodosum*, on a selection of bacteria relevant to gastrointestinal health. The chosen seaweed extracts were tested against two Gram + (*Staphylococcus aureus* and *Enterococcus faecalis*) and two Gram – bacteria (*Escherichia coli* and *Salmonella enterica*).

Staphylococcus aureus is a major opportunistic human pathogen which causes a variety of disease in clinical and community settings. Despite a wide variety of antimicrobial agents available, several strains of this species display resistance to antibiotics causing infection to spread. While *S. aureus* has not been identified as a causative agent of IBD, resistant strains such as methicillin resistant *Staphylococcus aureus* (MRSA) is a major concern for immune-compromised patients in clinical settings. Hospitalized patients with IBD are at increased risk of MRSA when compared with patients with non-IBD gastrointestinal issues and general medical inpatients (Nguyen *et al*., 2010). The introduction of natural anti-microbial agents either as a food component or as a natural supplement for IBD patients may act as a preventative measure against the establishment of such opportunistic infections. In this study all extracts tested inhibited the growth of *S. aureus* to differing degrees (MX121216 $\leq$ CC3689 < MX221216 < ETAUG1608). The *A. nodosum* extract (CC3689) and the Norwegian *S. latissima* extract (MX121216) had a wider inhibition range when compared with the other extracts indicating their suitability as an anti-microbial agent against *S. aureus*. When compared with the Norwegian *S. latissima* (MX121216), the Irish *S. latissima* only inhibited *S. aureus* growth at two concentrations (200mg/ml & 100mg/ml). As both extracts were extracted using water as a solvent and source material were harvested in the same season, origin of the source seaweed material may be a contributing factor to the differences in anti-microbial activity.

Similar inhibitory results on the growth of *E. faecalis* were observed with *A. nodosum* and Norwegian *S. latissima* demonstrating more of an inhibitory effect when compared with *A. esculenta* and Irish *S. latissima*. Increased abundance of the *Enterobacteriaceae* has been associated with inflammatory gastrointestinal disorders such as IBD (Lupp *et al*., 2007). Increased levels of *Enterococcus faecalis*, a common intestinal microbe, have been associated with clinically active Crohn's disease (Zhou *et al*., 2016). It has also been suggested through the use of animal models, that *E. faecalis* may play a causative

role in the development of IBD in genetically susceptible hosts (Balish & Warner, 2002). Due to the bacteriostatic activity of these extracts, the use of *A. nodosum*, *S. latissima* and *A. esculenta* extracts as anti-microbial agents may be a novel method for maintaining the growth levels of this potentially detrimental bacterium in diseased states. Smith *et al*., (2011) found that dietary supplementation with laminarin from *Laminarin digitata* in pigs with LPS induced colitis reduced populations of *Enterobacteriaceae* and enhanced cytokine release.

Both *E. coli* and *S. enterica* play a role in gastrointestinal health. Typically strains of *E. coli* can be found as natural components of the gut microbiota. However, a group of mucosal associated *E. coli* strains called the adherent and invasive *E. coli* (AIEC) have been isolated from newly diagnosed patients with Crohn's disease and ulcerative colitis which suggests that this group of bacteria may play a role in the early onset of these disorders (Sepehri *et al*., 2011). While the *E. coli* strain used in this study was not a member of this group, future research could utilise the inhibitory activity of the seaweed extracts against these AIEC in order to prevent onset of these inflammatory disorders. In the case of *S. enterica*, increased risk of developing IBD has been reported in individuals post *Salmonella* induced gastroenteritis (Rodríguez *et al*., 2006). IBD patients receiving immunomodulators are also at a greater risk of infection from this species due to supressed immune reactions. Recommended treatment of this type of infection would be the introduction of antibiotics and to withhold immunomodulatory therapy until infection is resolved, although re-infection could occur (Rahier *et al*., 2009). Therefore, the use of natural compounds such as seaweed bioactives with both anti-inflammatory activities (as seen in previous study) and anti-microbial activity against *Salmonella* species could be incorporated into treatments for those with IBD.

Both Norwegian and Irish *S. latissima*, as well as the *A. esculenta*, were the most effective at inhibiting growth of *E. coli* and *S. enterica* with the *A. nodosum* extract identified as least effective (MX121216 $\leq$ ETAUG1608 < MX221216 < CC3689). This may indicates that the variation between inhibitory activity of the *S. latissima* extracts observed in the Gram + bacteria may be due to composition of the extracts, rather than origin of seaweed material. As the extracts used in this study were crude extracts, the exact composition is unclear. However, it is reasonable to assume from results obtained from Gram + bacteria *S. aureus* and *E. faecalis*, that the Irish *S. latissima* contained lower levels of effective bioactives when compared with Norwegian *S. latissima*. Therefore Gram – bacteria such as *E. coli* and *S. enterica* may be more sensitive to

bioactives from *S. latissima* while Gram + bacteria such as *S. aureus* and *E.faecalis* are more sensitive to the *A. nodosum* extract.

Composition for seaweed bioactives are often affected by variations in the extraction method. Due to the chemical nature of seaweed bioactives, extraction requires optimization in each case in order to ensure maximum production of antimicrobials. Both *S. latissima* samples were extracted under similar conditions using water as a solvent. However, a potential variation between methods may be by the use of dry material (Irish *S. latissima*) vs wet material (Norwegian *S. latissima*). Drying is an important step in the extraction process as loss of valued antimicrobials could occur at high temperatures. Cox *et al*., (2012) found that extracts from fresh *Himanthalia elongata* achieved the highest inhibition against a number of foodborne pathogens when compared with extracts from dried *H. elongata*. Similarly Shanmughapriya *et al*., (2008) found that the drying process removed the antimicrobial capabilities of several species of seaweed. Therefore, in order to achieve highest antimicrobial activity, use of fresh seaweed material is recommended.

A potential barrier towards the use of these extracts is the possible detrimental effects against the host's natural flora. Therefore, the antimicrobial effects of these extracts were determined against *Lactobacillus johnsonii*, a probiotic strain which resides in the intestine. The highest concentrations of Norwegian *S. latissima* and *A. esculenta* (200mg/ml – 50mg/ml) demonstrated growth inhibitory activity against *L. johnsonii*. As this range contains the most effective inhibitory concentrations against chosen pathogenic bacteria this would indicate that in order to maintain balance of the microbial community in the gut, treatment using these extracts would require the addition of a probiotic. Bacteriostatic activity of extracts may also be an advantage in this case as re-growth of affected host bacteria may restore normal composition of gut flora. However, more in depth research in order to determine potential antimicrobial effects on additional members of the gut microbiota are required.

The highest concentration of the Irish *S. latissima* also inhibited the growth of *L. johnsonii*. However, lower concentrations (100-25mg/ml) of the Irish *S.latissima* extract and the *A. nodosum* extract promoted the growth of the *L. johnsonii* demonstrating prebiotic activity. These prebiotic effects may indicate a high concentration of algal polysaccharides in the extracts. Algal polysaccharides have been marked as potential prebiotics due to their ability to selectively promote the growth of beneficial bacteria

while inhibiting the growth of pathogenic strains. For example, sulphated polysaccharides from *Laminarin japonica* and *E.prolifera* fermented by faecal cultures were found to promote growth of Lactobacillus and Bifidobacterium species (Kong *et al*., 2016). The use of prebiotics has been highlighted as an emerging therapy for IBD and other gastrointestinal inflammatory disorders (Langen & Dieleman, 2009). While several substances have claimed to be prebiotics, so far only fructo-oligosaccharides, inulin, galacto-oligosaccharide and lactulose have met the criteria required for prebiotics (non-digestible, selective and fermentative ability). Therefore, further studies are required to investigate the non-digestibility, fermentative ability and selectivity of these extracts in order to assess their suitability as prebiotics.

The findings of the present study indicate that extracts from *Saccharina latissima*, *Alaria esculenta* and *Ascohyllum nodosum* have demonstrated bacteriostatic properties against a number of bacteria to differing degrees. These antimicrobial properties could be utilised in the maintenance of gastrointestinal health through the prevention of opportunistic infections and by monitoring the growth levels of detrimental bacteria such as *E. coli* and *E. faecalis* in diseased states. A barrier towards the use of these extracts as a natural antimicrobial supplement is their potential detrimental effects on the composition of the gut microbiota. While prebiotic effects were observed at lower concentrations of *S. latissima* and *A. nodosum*, further research is required to test the antimicrobial efficacy of these extracts using *in-vitro* models of the gut microbiota or through the use of animal trials.

Conclusions

With rising global incidences of gastrointestinal disorders such as inflammatory bowel disease (IBD), the use of novel therapeutic compounds to treat these disorders is of interest to the pharmaceutical industry. Current treatment methods of IBD typically include the use immune of supressing agents such as Anti-TNF compounds. However, not all patients respond to this course of treatment and adverse side effects such as increased susceptibility to infections and potential gastrointestinal malignancies are a concern. Therefore, the identification of a novel anti-inflammatory compound with anti-microbial activities could be of interest to the pharmaceutical industry either as a therapeutic or as a natural supplement. Seaweeds, in particular brown seaweed, are compelling sources of novel bioactive compounds which has implications for many chronic non-communicable diseases of the gastrointestinal tract, such as IBD.

The aims of this thesis were to assess both the anti-inflammatory and anti-microbial properties of extracts from four brown seaweed species *Saccharina latissima, Alaria esculenta, Ascophyllum nodosum* and *Fucus vesiculosus*. *S. latissima, A. esculenta* and *A. nodosum* demonstrated potent inhibitory activities against the production of Interleukin-8 (IL-8), which is a chemotactic cytokine highly expressed in patients with IBD. These anti-inflammatory activities have been attributed to their suppressive effects on IL-8 mRNA expression. However, as these extracts also reduced IL-8 production in normal conditions, intake of these extracts should be limited to those suffering gastrointestinal inflammation. Extracts from *S. latissima, A. esculenta* and *A. nodosum* also demonstrated bacteriostatic activities to differing degrees against a number of pathogenic bacteria including *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Salmonella enterica,* which could prevent the establishment of opportunistic infections. However, at 25mg/ml, which is the chosen effective anti-inflammatory concentration assessed in the *in-vitro* model, no consistent anti-microbial activity across all extracts was determined, which implies that effective anti-microbial concentration needed to compliment the anti-inflammatory activities is dependent on the extract selected. Further work using animal or clinical trials is required to assess whether anti-inflammatory and anti-microbial activities of these extracts are applicable *in vivo.*

Bibliography

Albert, B.B. et al., 2014. Higher omega-3 index is associated with increased insulin sensitivity and more favourable metabolic profile in middle-aged overweight men. *Scientific reports*, 4, p.6697. Available at: http://www.nature.com/srep/2014/141021/srep06697/full/srep06697.html.

Almela, C. et al., 2006. Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. *Food and Chemical Toxicology*, 44(11), pp.1901–1908.

Andersen, G.S., Pedersen, M.F. & Nielsen, S.L., 2013. Temperature acclimation and heat tolerance of photosynthesis in Norwegian Saccharina latissima (Laminariales, Phaeophyceae). *Journal of Phycology*, 49(4), pp.689–700.

Anderson, J.W. et al., 2009. Health benefits of dietary fiber. *Nutrition Reviews*, 67(4), pp.188–205.

Anis, M., Ahmed, S. & Hasan, M.M., 2017. Algae As Nutrition, Medicine and Cosmetic: the Forgotten History, Present Status and Future Trends. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6(6), pp.1934–1959. Available at: http://wjpps.com/wjpps_controller/abstract_id/7266.

Awang, A.N. et al., 2013. Anti-obesity property of the brown seaweed, Sargassum polycystum using an in vivo animal model. *Journal of Applied Phycology*, pp.1–6.

Axelrad, J.E., Lichtiger, S. & Yajnik, V., 2016. Inflammatory bowel disease and cancer: The role of inflammation, immunosuppression, and cancer treatment. *World Journal of Gastroenterology*, 22(20), pp.4794–4801.

Balina, K., Romagnoli, F. & Blumberga, D., 2017. Seaweed biorefinery concept for sustainable use of marine resources. *Energy Procedia*, 128, pp.504–511. Available at: http://linkinghub.elsevier.com/retrieve/pii/S1876610217339115.

Balish, E. & Warner, T., 2002. Enterococcus faecalis induces inflammatory bowel disease in interleukin-10 knockout mice. *The American journal of pathology*, 160(6), pp.2253–2257.

Bassaganya-Riera, J. et al., 2011. Soluble Fibers and Resistant Starch Ameliorate Disease Activity in Interleukin-10 – Deficient Mice with Inflammatory Bowel

Disease. *The Journal of nutrition*, 141(6), pp.1318–1325.

Bleakley, S. & Hayes, M., 2017. Algal Proteins: Extraction, Application, and Challenges Concerning Production. *Foods*, 6(5), p.33. Available at: http://www.mdpi.com/2304-8158/6/5/33.

Boulangé, C.L. et al., 2016. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. *Genome Medicine*, 8(1), pp.1–12. Available at: http://dx.doi.org/10.1186/s13073-016-0303-2.

Brown, E.M. et al., 2014. Seaweed and human health. *Nutrition Reviews*, 72(3), pp.205–216.

Brul, S. & Coote, P., 1999. Preservative agents in foods: Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology*, 50(1–2), pp.1–17.

Bu, S.Y., Kwon, H. & Sung, M., 2014. Supplementation of Seaweeds Extracts Suppresses Azoxymethane-induced Aberrant DNA Methylation in Colon and Liver of ICR Mice. *Journal of Cancer Prevention*, 19(3), pp.216–223.

Buchholz, C.M., Krause, G. & Buck, B.H., 2012. Seaweed Biology. *Seaweed Biology*, 219, pp.471–493. Available at: http://www.springerlink.com/index/10.1007/978-3-642-28451-9.

Buchholz, T. & Melzig, M.F., 2015. Polyphenolic Compounds as Pancreatic Lipase Inhibitors. *Planta Medica*, 81(10), pp.771–783.

Černá, M., 2011. *Seaweed proteins and amino acids as nutraceuticals* 1st ed., Elsevier Inc. Available at: http://dx.doi.org/10.1016/B978-0-12-387669-0.00024-7.

Di Cesare, M. et al., 2016. Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *The Lancet*, 387(10026), pp.1377–1396. Available at: http://dx.doi.org/10.1016/S0140-6736(16)30054-X.

Chater, P.I. et al., 2016. Inhibitory activity of extracts of Hebridean brown seaweeds on lipase activity. *Journal of Applied Phycology*, 28(2), pp.1303–1313.

Chin, Y.X. et al., 2015. Anti-diabetic potential of selected Malaysian seaweeds. *Journal*

of Applied Phycology, 27(5), pp.2137–2148.

Chouraki, V. et al., 2011. The changing pattern of Crohn's disease incidence in northern France: A continuing increase in the 10- to 19-year-old age bracket (1988-2007). *Alimentary Pharmacology and Therapeutics*, 33(10), pp.1133–1142.

Connan, S. & Stengel, D.B., 2011. Impacts of ambient salinity and copper on brown algae: 1. Interactive effects on photosynthesis, growth, and copper accumulation. *Aquatic Toxicology*, 104(1–2), pp.94–107.

Corcoran, J., O'shea, H. & McGlynn, H., 2014. Harvest Atlantic Project sectorial analysis of marine biotechnology in the Atlantic Area. *Journal of Maritime Research*, 11(I), pp.81–85.

Corona, G. et al., 2016. Gastrointestinal modifications and bioavailability of brown seaweed phlorotannins and effects on inflammatory markers. *British Journal of Nutrition*, 115(7), pp.1240–1253. Available at: http://www.journals.cambridge.org/abstract_S0007114516000210.

Corridoni, D., O. Arseneau, K. & Cominelli, F., 2014. Inflammatory bowel disease. *Immunology Letters*, 161(10), pp.231–235. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11105426.

Cox, S., 2010. An Assessment of the Antioxidant and Antimicrobial Activity of Six Species of Edible Irish Seaweeds. *International Food Research Journal*, 17, pp.205–220.

Cox, S., Abu-Ghannam, N. & Gupta, S., 2012. *Effect of processing conditions on phytochemical constituents of edible irish seaweed himanthalia elongata*,

Cruces, E., Huovinen, P. & Gómez, I., 2013. Interactive effects of UV radiation and enhanced temperature on photosynthesis, phlorotannin induction and antioxidant activities of two sub-Antarctic brown algae. *Marine Biology*, 160(1), pp.1–13.

Cumashi, A. et al., 2007. A comparative study of the anti-inflammatory, anticoagulant, antiangiogenic, and antiadhesive activities of nine different fucoidans from brown seaweeds. *Glycobiology*, 17(5), pp.541–552.

Dar, A. et al., 2007. Effect of seasonal variation on the anti-inflammatory activity of Sargassum wightii growing on the N. Arabian Sea coast of Pakistan. *Journal of*

Experimental Marine Biology and Ecology, 351(1–2), pp.1–9.

Das, S.K. et al., 2005. Fucoxanthin induces cell cycle arrest at G0/G1 phase in human colon carcinoma cells through up-regulation of p21WAF1/Cip1. *Biochimica et Biophysica Acta - General Subjects*, 1726(3), pp.328–335.

Dawczynski, C., Schubert, R. & Jahreis, G., 2007. Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chemistry*, 103(3), pp.891–899.

Devillé, C. et al., 2004. Laminarin in the dietary fibre concept. *Journal of the Science of Food and Agriculture*, 84(9), pp.1030–1038.

Devillé, C. et al., 2007. Study on the effects of laminarin, a polysaccharide from seaweed, on gut characteristics. *Journal of the Science of Food and Agriculture*, 87(2), pp.1717–1725.

Dillehay, T.D. et al., 2015. New archaeological evidence for an early human presence at Monte Verde, Chile. *PLoS ONE*, 10(11), pp.1–27.

Dring, M., Edwards, M. & Watson, L., 2013. Development and Demonstration of Viable Hatchery and Ongoing Methodologies for Seaweed Species with Identified Commercial Potential. , pp.1–31.

Ehrig, K. & Alban, S., 2015. Sulfated galactofucan from the brown alga saccharina latissima-variability of yield, structural composition and bioactivity. *Marine Drugs*, 13(1), pp.76–101.

Eilander, A., Harika, R.K. & Zock, P.L., 2015. Intake and sources of dietary fatty acids in Europe: Are current population intakes of fats aligned with dietary recommendations? *European Journal of Lipid Science and Technology*, 117(9), pp.1370–1377.

Eom, S.H., Kim, Y.M. & Kim, S.K., 2012. Antimicrobial effect of phlorotannins from marine brown algae. *Food and Chemical Toxicology*, 50(9), pp.3251–3255. Available at: http://dx.doi.org/10.1016/j.fct.2012.06.028.

European Food Safety Authority, 2015. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2014. *EFSA Journal*, 13(12). Available at: http://doi.wiley.com/10.2903/j.efsa.2015.4329.

FAO, 2017. An Overview of Recently Published Global Aquaculture Statistics.

FAO, 2015. *World Aquaculture 2015 : a Brief Overview*, Available at: http://www.fao.org/3/a-i7546e.pdf.

Farthing, M. et al., 2014. Survey of digestive health across Europe: Final report. Part 1: The burden of gastrointestinal diseases and the organisation and delivery of gastroenterology services across Europe. *United European Gastroenterology Journal*, 2(6), pp.539–543. Available at: http://ueg.sagepub.com/lookup/doi/10.1177/2050640614554154.

Ferlay, J. et al., 2015. Reprint of: Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. *European Journal of Cancer*, 51(9), pp.1201–1202. Available at: http://dx.doi.org/10.1016/j.ejca.2012.12.027.

Fleurence, È., Physico-chimie, Â. & Qualite, L., 1999. Seaweed proteins: biochemical, nutritional and potential uses. *Trends in Food Science & Technology*, 10, pp.25–28.

Flores, S.R.L., Dobbs, J. & Dunn, M.A., 2015. Mineral nutrient content and iron bioavailability in common and Hawaiian seaweeds assessed by an in vitro digestion/Caco-2 cell model. *Journal of Food Composition and Analysis*, 43, pp.185–193. Available at: http://www.sciencedirect.com/science/article/pii/S0889157515001507.

Ford, A.C. & Peyrin-Biroulet, L., 2013. Opportunistic Infections With Anti-Tumor Necrosis Factor-α Therapy in Inflammatory Bowel Disease: Meta-Analysis of Randomized Controlled Trials. *The American Journal of Gastroenterology*, 108(8), pp.1268–1276. Available at: http://www.nature.com/doifinder/10.1038/ajg.2013.138.

Førde, H. et al., 2016. Development of bryozoan fouling on cultivated kelp (Saccharina latissima) in Norway. *Journal of Applied Phycology*, 28(2), pp.1225–1234.

van Ginneken, V.J.T. et al., 2011. Polyunsaturated fatty acids in various macroalgal species from North Atlantic and tropical seas. *Lipids in health and disease*, 10(1), p.104. Available at: http://www.lipidworld.com/content/10/1/104%5Cnhttp://lipidworld.biomedcentral.com/articles/10.1186/1476-511X-10-104.

Gómez-Ordóñez, E., Jiménez-Escrig, A. & Rupérez, P., 2010. Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Research International*, 43, pp.2289–2294. Available at: http://eprints.ucm.es/20162/.

Goss, R. & Jakob, T., 2010. Regulation and function of xanthophyll cycle-dependent photoprotection in algae. *Photosynthesis research*, 106(1–2), pp.103–22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/20224940.

Guariguata, L. et al., 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*, 103(2), pp.137–149. Available at: http://dx.doi.org/10.1016/j.diabres.2013.11.002.

Gupta, S. et al., 2012. Growth Inhibition of Common Food Spoilage and Pathogenic Microorganisms in the Presence of Brown Seaweed Extracts. *Food and Bioprocess Technology*, 5(5), pp.1907–1916.

Gupta, S. & Abu-Ghannam, N., 2011a. Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science and Technology*, 22(6), pp.315–326. Available at: http://dx.doi.org/10.1016/j.tifs.2011.03.011.

Gupta, S. & Abu-Ghannam, N., 2011b. Recent developments in the application of seaweeds or seaweed extracts as a means for enhancing the safety and quality attributes of foods. *Innovative Food Science and Emerging Technologies*, 12(4), pp.600–609. Available at: http://dx.doi.org/10.1016/j.ifset.2011.07.004.

Gupta, S., Rajauria, G. & Abu-Ghannam, N., 2010. Study of the microbial diversity and antimicrobial properties of Irish edible brown seaweeds. *International Journal of Food Science and Technology*, 45(3), pp.482–489.

Hanauer, S.B. et al., 2002. Maintenance infliximab for Crohn's disease: The ACCENT I randomised trial. *Lancet*, 359(9317), pp.1541–1549.

Handå, A. et al., 2013. Seasonal- and depth-dependent growth of cultivated kelp (Saccharina latissima) in close proximity to salmon (Salmo salar) aquaculture in Norway. *Aquaculture*, 414–415(February), pp.191–201. Available at: http://dx.doi.org/10.1016/j.aquaculture.2013.08.006.

Heo, S.J. et al., 2012. Anti-inflammatory effect of fucoxanthin derivatives isolated from

Sargassum siliquastrum in lipopolysaccharide-stimulated RAW 264.7 macrophage. *Food and Chemical Toxicology*, 50(9), pp.3336–3342. Available at: http://dx.doi.org/10.1016/j.fct.2012.06.025.

Heo, S.J. et al., 2010. Evaluation of anti-inflammatory effect of fucoxanthin isolated from brown algae in lipopolysaccharide-stimulated RAW 264.7 macrophages. *Food and Chemical Toxicology*, 48(8–9), pp.2045–2051. Available at: http://dx.doi.org/10.1016/j.fct.2010.05.003.

Horie, Y., Sugase, K. & Horie, K., 1995. Physiological differences of soluble and insoluble dietary fibre fractions of brown algae and mushrooms in pepsin activity in vitro and protein digestibility. *Asia Pacific journal of clinical nutrition*, 4(2), pp.251–255.

Hoshiyama, Y., Sekine, T. & Sasaba, T., 1993. A case-control study of colorectal cancer and its relation to diet, cigarettes and alcohol consumption in Saitama prefecture, Japan. *The Tohoku Journal of Experimental Medicine*, 171(2), pp.153–165.

Hosokawa, M. et al., 2004. Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARg ligand, troglitazone, on colon cancer cells. *Biochimica et Biophysica Acta - General Subjects*, 1675(1–3), pp.113–119.

Houghton, D. et al., 2015. Biological activity of alginate and its effect on pancreatic lipase inhibition as a potential treatment for obesity. *Food Hydrocolloids*, 49, pp.18–24. Available at: http://dx.doi.org/10.1016/j.foodhyd.2015.02.019.

Hurst, D., 2013. *Marine Biotechnology Strategic Research and Innovation ROADMAP*,

Hwang, E.S. & Thi, N. Do, 2014. Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (Porphyra tenera). *Preventive Nutrition and Food Science*, 19(1), pp.40–48.

Hwang, P.A. et al., 2015. The brown seaweed Sargassum hemiphyllum exhibits ??-amylase and ??-glucosidase inhibitory activity and enhances insulin release in vitro. *Cytotechnology*, 67(4), pp.653–660. Available at: http://dx.doi.org/10.1007/s10616-014-9745-9.

Hyun, J.-H. et al., 2009. Apoptosis inducing activity of fucoidan in HCT-15 colon

carcinoma cells. *Biological & pharmaceutical bulletin*, 32(10), pp.1760–1764.

Irving, P.M. & Gibson, P.R., 2008. Infections and IBD. *Nature Clinical Practice Gastroenterology and Hepatology*, 5(1), pp.18–27.

Islam, M.N. et al., 2013. Anti-inflammatory activity of edible brown alga Saccharina japonica and its constituents pheophorbide a and pheophytin a in LPS-stimulated RAW 264.7 macrophage cells. *Food and Chemical Toxicology*, 55, pp.541–548. Available at: http://dx.doi.org/10.1016/j.fct.2013.01.054.

Jahns, P., Latowski, D. & Strzalka, K., 2009. Mechanism and regulation of the violaxanthin cycle: The role of antenna proteins and membrane lipids. *Biochimica et Biophysica Acta - Bioenergetics*, 1787(1), pp.3–14. Available at: http://dx.doi.org/10.1016/j.bbabio.2008.09.013.

Jensen, M.G., Kristensen, M. & Astrup, A., 2012. Effect of alginate supplementation on weight loss in obese subjects completing a 12-wk energy-restricted diet: A randomized controlled trial. *American Journal of Clinical Nutrition*, 96(1), pp.5–13.

Jess, T., Rungoe, C. & Peyrin-Biroulet, L., 2012. Risk of Colorectal Cancer in Patients With Ulcerative Colitis: A Meta-analysis of Population-Based Cohort Studies. *Clinical Gastroenterology and Hepatology*, 10(6), pp.639–645. Available at: http://dx.doi.org/10.1016/j.cgh.2012.01.010.

Jiang, Z. et al., 2010. Effects of sulfated fucan, ascophyllan, from the brown Alga Ascophyllum nodosum on various cell lines: A comparative study on ascophyllan and fucoidan. *Journal of Bioscience and Bioengineering*, 110(1), pp.113–117. Available at: http://dx.doi.org/10.1016/j.jbiosc.2010.01.007.

Jung, H.A. et al., 2014. Phlorotannins isolated from the edible brown alga Ecklonia stolonifera exert anti-adipogenic activity on 3T3-L1 adipocytes by downregulating C/EBPα and PPARγ. *Fitoterapia*, 92(July 2015), pp.260–269. Available at: http://dx.doi.org/10.1016/j.fitote.2013.12.003.

Kaczmarczyk, M.M., Miller, M.J. & Freund, G.G., 2012. The health benefits of dietary fiber: Beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. *Metabolism: Clinical and Experimental*, 61(8), pp.1058–1066. Available at: http://dx.doi.org/10.1016/j.metabol.2012.01.017.

Kahn, S.E., Hull, R.L. & Utzschneider, K.M., 2006. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*, 444(7121), pp.840–846. Available at: http://www.nature.com/doifinder/10.1038/nature05482.

Khan, M.N.. et al., 2008. Anti-inflammatory activites of methanol extracts from various seaweed species. *Journal of Environmental Biology*, 29(July 2008), pp.465–469.

Kim, E.J. et al., 2010. Fucoidan present in brown algae induces apoptosis of human colon cancer cells. *BMC gastroenterology*, 10, p.96.

Kim, K.J., Lee, O.H. & Lee, B.Y., 2010. Fucoidan, a sulfated polysaccharide, inhibits adipogenesis through the mitogen-activated protein kinase pathway in 3T3-L1 preadipocytes. *Life Sciences*, 86(21–22), pp.791–797. Available at: http://dx.doi.org/10.1016/j.lfs.2010.03.010.

Kim, K.N. et al., 2010. Fucoxanthin inhibits the inflammatory response by suppressing the activation of NF-??B and MAPKs in lipopolysaccharide-induced RAW 264.7 macrophages. *European Journal of Pharmacology*, 649(1–3), pp.369–375. Available at: http://dx.doi.org/10.1016/j.ejphar.2010.09.032.

Kim, K.T., Rioux, L.E. & Turgeon, S.L., 2014. Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by fucoidan obtained from Fucus vesiculosus and Ascophyllum nodosum. *Phytochemistry*, 98, pp.27–33. Available at: http://dx.doi.org/10.1016/j.phytochem.2013.12.003.

Kim, M.S. et al., 2008. Effects of seaweed supplementation on blood glucose concentration, lipid profile, and antioxidant enzyme activities in patients with type 2 diabetes mellitus. *Nutrition research and practice*, 2(2), pp.62–7. Available at: http://synapse.koreamed.org/DOIx.php?id=10.4162/nrp.2008.2.2.62.

Kong, Q. et al., 2016. In vitro fermentation of sulfated polysaccharides from E. prolifera and L. japonica by human fecal microbiota. *International Journal of Biological Macromolecules*, 91, pp.867–871. Available at: http://dx.doi.org/10.1016/j.ijbiomac.2016.06.036.

Kong, Z. et al., 2016. Fucoxanthin-Rich Brown Algae Extract Decreases Inflammation and Attenuates Colitis-associated Colon Cancer in Mice. *Journal of Food and Nutrition Research*, 4(3), pp.137–147.

Koyanagi, S. et al., 2003. Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. *Biochemical Pharmacology*, 65(2), pp.173–179.

Krumhansl, K.A., Lee, J.M. & Scheibling, R.E., 2011. Grazing damage and encrustation by an invasive bryozoan reduce the ability of kelps to withstand breakage by waves. *Journal of Experimental Marine Biology and Ecology*, 407(1), pp.12–18. Available at: http://dx.doi.org/10.1016/j.jembe.2011.06.033.

Kucharzik, T. et al., 2005. Acute induction of human IL-8 production by intestinal epithelium triggers neutrophil infiltration without mucosal injury. *Gut*, 54(11), pp.1565–1572.

Kumar, M. et al., 2014. *Salinity and desiccation induced oxidative stress acclimation in seaweeds*, Elsevier. Available at: http://dx.doi.org/10.1016/B978-0-12-408062-1.00004-4.

Kumar, S.A. et al., 2015. Seaweed supplements normalise metabolic, cardiovascular and liver responses in high-carbohydrate, high-fat fed rats. *Marine Drugs*, 13(2), pp.788–805.

Langen, M. & Dieleman, L.A., 2009. Prebiotics in Chronic Intestinal Inflammation. *Inflammatory Bowel Diseases*, 15(3), pp.454–462.

Larsen, N. et al., 2010. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS ONE*, 5(2).

Larussa, T., Imeneo, M. & Luzza, F., 2017. Potential role of nutraceutical compounds in inflammatory bowel disease. *World Journal of Gastroenterology*, 23(14), pp.2483–2492.

Lean, Q.Y. et al., 2015. Fucoidan Extracts Ameliorate Acute Colitis. *PloS one*, 10(6), p.e0128453. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4471193&tool=pmcentrez&rendertype=abstract.

Lee, H.J. et al., 2010. Algae consumption and risk of type 2 diabetes: Korean National Health and Nutrition Examination Survey in 2005. *Journal of nutritional science and vitaminology*, 56, pp.13–18.

Leonard, S.G. et al., 2011. Effects of dietary seaweed extract supplementation in sows

and post-weaned pigs on performance, intestinal morphology, intestinal microflora and immune status. *British Journal of Nutrition*, 106(5), pp.688–699.

Libby, P., Ridker, P.M. & Maseri, A., 2002. Inflammation and atherosclerosis. *Circulation*, 105(9), pp.1135–1143.

Liu, M. et al., 2017. Antibacterial activity and mechanisms of depolymerized fucoidans isolated from Laminaria japonica. *Carbohydrate Polymers*, 172, pp.294–305. Available at: http://dx.doi.org/10.1016/j.carbpol.2017.05.060.

Longobardi, T., Jacobs, P. & Bernstein, C.N., 2004. Utilization of health care resources by individuals with inflammatory bowel disease in the United States: a profile of time since diagnosis. *The American journal of gastroenterology*, 99(4), pp.650–5. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15089897.

Lopes, G., Andrade, P. & Valentão, P., 2016. Phlorotannins: Towards New Pharmacological Interventions for Diabetes Mellitus Type 2. *Molecules*, 22(1), p.56. Available at: http://www.mdpi.com/1420-3049/22/1/56.

Lordan, S. et al., 2013. The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. *Food Chemistry*, 141(3), pp.2170–2176.

Lunagariya, N.A. et al., 2014. Inhibitors of pancreatic lipase: State of the art and clinical perspectives. *EXCLI Journal*, 13, pp.897–921.

Lupp, C. et al., 2007. Host-Mediated Inflammation Disrupts the Intestinal Microbiota and Promotes the Overgrowth of Enterobacteriaceae. *Cell Host and Microbe*, 2(2), pp.119–129.

Macarthur, M., Hold, G.L. & El-Omar, E.M., 2004. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. *Am.J.Physiol Gastrointest.Liver Physiol*, 286(0193–1857 (Print)), pp.G515–G520.

Maeda, H. et al., 2006. Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *International Journal of Molecular Medicine*, 18(1), pp.147–152.

Marinho, G.S. et al., 2015. Lipids and composition of fatty acids of Saccharina latissima cultivated year-round in integrated multi-trophic aquaculture. *Marine Drugs*, 13(7),

pp.4357–4374.

Matanjun, P. et al., 2008. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *Journal of Applied Phycology*, 20(4), pp.367–373.

Matsumoto, M. et al., 2010. Suppressive effects of the marine carotenoids, fucoxanthin and fucoxanthinol on triglyceride absorption in lymph duct-cannulated rats. *European Journal of Nutrition*, 49(4), pp.243–249.

Matsuoka, K. & Kanai, T., 2015. The gut microbiota and inflammatory bowel disease. *Seminars in Immunopathology*, 37(1), pp.47–55.

McCormack, G. et al., 2001. Tissue cytokine and chemokine expression in inflammatory bowel disease. *Inflammation Research*, 50(10), pp.491–495.

Mitsuyama, K. et al., 1994. IL-8 as an important chemoattractant for neutrophils in ulcerative colitis and Crohn's disease. *Clinical and experimental immunology*, 96(3), pp.432–6. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1534558&tool=pmcentrez&rendertype=abstract.

Molodecky, N.A. et al., 2012. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology*, 142(1), p.46–54.e42. Available at: http://dx.doi.org/10.1053/j.gastro.2011.10.001.

Mac Monagail, M. et al., 2017. Sustainable harvesting of wild seaweed resources. *European Journal of Phycology*, 52(4), pp.371–390. Available at: https://doi.org/10.1080/09670262.2017.1365273.

Monk, J.M. et al., 2012. Dietary n-3 Polyunsaturated Fatty Acids (PUFA) Decrease Obesity-Associated Th17 Cell-Mediated Inflammation during Colitis. *PLoS ONE*, 7(11), pp.1–10.

Moriguch, E.H., Moriguchi, Y. & Yamori, Y., 2004. Impact of diet on the cardiovascular risk profile of Japanese immigrants li. *Clinical and Experimental Pharmacology and Physiology*, 31, pp.S5–S7.

Morrissey, K., O'Donoghue, C. & Hynes, S., 2011. Quantifying the value of multi-sectoral marine commercial activity in Ireland. *Marine Policy*, 35(5), pp.721–727. Available at: http://dx.doi.org/10.1016/j.marpol.2011.02.013.

Müller, R., Wiencke, C. & Bischof, K., 2008. Interactive effects of UV radiation and temperature on microstages of Laminariales (Phaeophyceae) from the Arctic and North Sea. *Climate Research*, 37(2–3), pp.203–213.

Nelson, M.M., Phleger, C.F. & Nichols, P.D., 2002. Seasonal lipid composition in macroalgae of the northeastern Pacific Ocean. *Botanica Marina*, 45(1), pp.58–65.

Ng, M. et al., 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*, 384. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24880830 [Accessed July 10, 2014].

Nguyen, G.C., Patel, H. & Chong, R.Y., 2010. Increased prevalence of and associated mortality with methicillin-resistant staphylococcus aureus among hospitalized IBD patients. *American Journal of Gastroenterology*, 105(2), pp.371–377. Available at: http://dx.doi.org/10.1038/ajg.2009.581.

Nobili, S. et al., 2009. Natural compounds for cancer treatment and prevention. *Pharmacological Research*, 59(6), pp.365–378.

O'Gorman, D.M., Tierney, C.M., et al., 2012. The marine-derived, multi-mineral formula, aquamin, enhances mineralisation of osteoblast cells in vitro. *Phytotherapy Research*, 26(3), pp.375–380.

O'Gorman, D.M., O'Carroll, C. & Carmody, R.J., 2012. Evidence that marine-derived, multi-mineral, aquamin inhibits the NF-κB signaling pathway in vitro. *Phytotherapy Research*, 26(4), pp.630–632.

O'Shea, C.J. et al., 2016. The effect of algal polysaccharides laminarin and fucoidan on colonic pathology, cytokine gene expression and Enterobacteriaceae in a dextran sodium sulfate-challenged porcine model. *Journal of Nutritional Science*, 5(6), p.e15. Available at: http://www.journals.cambridge.org/abstract_S2048679016000045.

O'Sullivan, A.M. et al., 2011. In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. *Food Chemistry*, 126(3), pp.1064–1070. Available at: http://dx.doi.org/10.1016/j.foodchem.2010.11.127.

Okamoto, R., 2011. Epithelial regeneration in inflammatory bowel diseases. *Inflammation and Regeneration*, 31(3), pp.275–281. Available at: http://joi.jlc.jst.go.jp/JST.JSTAGE/inflammregen/31.275?from=CrossRef.

Ortiz, J. et al., 2006. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds Ulva lactuca and Durvillaea antarctica. *Food Chemistry*, 99, pp.98–104.

Papi, C. et al., 2013. Mucosal healing in inflammatory bowel disease: Treatment efficacy and predictive factors. *Digestive and Liver Disease*, 45(12), pp.978–985. Available at: http://dx.doi.org/10.1016/j.dld.2013.07.006.

Park, H.Y. et al., 2011. Anti-inflammatory effects of fucoidan through inhibition of NF-κB, MAPK and Akt activation in lipopolysaccharide-induced BV2 microglia cells. *Food and Chemical Toxicology*, 49(8), pp.1745–1752. Available at: http://dx.doi.org/10.1016/j.fct.2011.04.020.

Patra, J.K. & Baek, K., 2016. Anti-Listerial Activity of Four Seaweed Essential Oils Against Listeria monocytogenes. *Jundishapur Journal of Microbiology*, 9(7).

Peng, L. et al., 2007. Effects of butyrate on intestinal barrier function in a caco-2 cell monolayer model of intestinal barrier. *Pediatric Research*, 61(1), pp.37–41.

Pérez, M.J., Falqué, E. & Domínguez, H., 2016. Antimicrobial action of compounds from marine seaweed. *Marine Drugs*, 14(3), pp.1–38.

Peters, H.P.F. et al., 2011. Dose-dependent suppression of hunger by a specific alginate in a low-viscosity drink formulation. *Obesity (Silver Spring, Md.)*, 19(6), pp.1171–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/21512509.

Pineton de Chambrun, G., Blanc, P. & Peyrin-Biroulet, L., 2016. Current evidence supporting mucosal healing and deep remission as important treatment goals for inflammatory bowel disease. *Expert Review of Gastroenterology and Hepatology*, 10(8), pp.915–927. Available at: http://dx.doi.org/10.1586/17474124.2016.1174064.

Praiboon, J. et al., 2017. Seasonal variation in nutritional composition and anti-proliferative activity of brown seaweed, Sargassum oligocystum. *Journal of Applied Phycology*, pp.1–11.

Psichas, a et al., 2015. The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *International Journal of Obesity*, 39(3), pp.424–429. Available at: http://www.nature.com/doifinder/10.1038/ijo.2014.153.

Rahier, J.F. et al., 2009. European evidence-based Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *Journal of Crohn's and Colitis*, 3(2), pp.47–91. Available at: http://dx.doi.org/10.1016/j.crohns.2009.02.010.

Rajapakse, N. & Kim, S.K., 2011. *Nutritional and digestive health benefits of seaweed* 1st ed., Elsevier Inc. Available at: http://dx.doi.org/10.1016/B978-0-12-387669-0.00002-8.

Rajauria, G. et al., 2010. Effect of hydrothermal processing on colour, antioxidant and free radical scavenging capacities of edible Irish brown seaweeds. *International Journal of Food Science and Technology*, 45(12), pp.2485–2493.

Rajauria, G. & Abu-Ghannam, N., 2013. Isolation and partial characterization of bioactive fucoxanthin from himanthalia elongata brown seaweed: A TLC-based approach. *International Journal of Analytical Chemistry*, (May 2013).

Ramnani, P. et al., 2012. Invitro fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. *Anaerobe*, 18(1), pp.1–6. Available at: http://dx.doi.org/10.1016/j.anaerobe.2011.08.003.

Robertson, R.C. et al., 2015. The anti-inflammatory effect of algae-derived lipid extracts on lipopolysaccharide (LPS)-stimulated human THP-1 macrophages. *Marine Drugs*, 13(8), pp.5402–5424.

Rodemann, J.F. et al., 2007. Incidence of Clostridium difficile Infection in Inflammatory Bowel Disease. *Clinical Gastroenterology and Hepatology*, 5(3), pp.339–344.

Rodrigues, D. et al., 2016. In vitro fermentation and prebiotic potential of selected extracts from seaweeds and mushrooms. *LWT - Food Science and Technology*, 73, pp.131–139. Available at: http://dx.doi.org/10.1016/j.lwt.2016.06.004.

Rodrigues, M.A. et al., 2002. A smaller and impaired xanthophyll cycle makes the deep sea macroalgae Laminaria abyssalis (Phaeophyceae) highly sensitive to daylight when compared with shallow water Laminaria digitata. *Journal of Phycology*, 38(5), pp.939–947.

Rodríguez, L.A.G., Ruigómez, A. & Panés, J., 2006. Acute Gastroenteritis Is Followed by an Increased Risk of Inflammatory Bowel Disease. *Gastroenterology*, 130(6), pp.1588–1594.

Rogler, G., 2010. Gastrointestinal and liver adverse effects of drugs used for treating IBD. *Best Practice and Research: Clinical Gastroenterology*, 24(2), pp.157–165. Available at: http://dx.doi.org/10.1016/j.bpg.2009.10.011.

Ruzickova, J. et al., 2004. Omega-3 PUFA of marine origin limit diet-induced obesity in mice by reducing cellularity of adipose tissue. *Lipids*, 39(12), pp.1177–1185.

Sachindra, N.M. et al., 2007. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *Journal of Agricultural and Food Chemistry*, 55(21), pp.8516–8522.

Sandsdalen, E. et al., 2003. The antibacterial effect of a polyhydroxylated fucophlorethol from the marine brown alga, Fucus vesiculosus. *World Journal of Microbiology and Biotechnology*, 19(8), pp.777–782.

Sanjeewa, K.K.A. et al., 2016. Bioactive properties and potentials cosmeceutical applications of phlorotannins isolated from brown seaweeds: A review. *Journal of Photochemistry and Photobiology B: Biology*, 162, pp.100–105. Available at: http://dx.doi.org/10.1016/j.jphotobiol.2016.06.027.

Scherner, F. et al., 2013. Salinity critical threshold values for photosynthesis of two cosmopolitan seaweed species: Providing baselines for potential shifts on seaweed assemblages. *Marine Environmental Research*, 91(May), pp.14–25.

Selvaraj, S. & Palanisamy, S., 2014. Investigations on the anti-diabetic potential of novel marine seaweed Sargassum longiotom against alloxan-induced diabetes mellitus: A pilot study. *Bangladesh Journal of Pharmacology*, 9(2), pp.194–197.

Sepehri, S. et al., 2011. Characterization of Escherichia coli isolated from gut biopsies of newly diagnosed patients with inflammatory bowel disease. *Inflammatory*

Bowel Diseases, 17(7), pp.1451–1463.

Shahidi, F., 2000. Antioxidants in food and food antioxidants. *Nahrung - Food*, 44(3), pp.158–163.

Shanmughapriya, S. et al., 2008. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Annals of Microbiology*, 58(3), pp.535–541.

Sharifuddin, Y. et al., 2015. Potential bioactive compounds from seaweed for diabetes management. *Marine Drugs*, 13(8), pp.5447–5491.

Shiratori, K. et al., 2005. Effects of fucoxanthin on lipopolysaccharide-induced inflammation in vitro and in vivo. *Experimental Eye Research*, 81(4), pp.422–428.

Siegel, R.L., Miller, K.D. & Jemal, A., 2016. Cancer statistics. *CA Cancer J Clin*, 66(1), pp.7–30.

Singh, P. et al., 2015. Intestinal microbial communities associated with acute enteric infections and disease recovery. *Microbiome*, 3, p.45. Available at: http://dx.doi.org/10.1186/s40168-015-0109-2.

Singh, R.P. et al., 2011. Isolation of seaweed-associated bacteria and their morphogenesis-inducing capability in axenic cultures of the green alga Ulva fasciata. *Aquatic Biology*, 12(1), pp.13–21.

Singh, R.P. & Reddy, C.R.K., 2014. Seaweed-microbial interactions: Key functions of seaweed-associated bacteria. *FEMS Microbiology Ecology*, 88(2), pp.213–230.

Skriptsova, A. V., 2016. Seasonal variations in the fucoidan content of brown algae from Peter the Great Bay, Sea of Japan. *Russian Journal of Marine Biology*, 42(4), pp.351–356. Available at: http://link.springer.com/10.1134/S1063074016040106.

Škrovánková, S., 2011. Seaweed vitamins as nutraceuticals. *Advances in Food and Nutrition Research*, 64, pp.357–369.

Smith, A.G. et al., 2011. The effects of laminarin derived from Laminaria digitata on measurements of gut health: Selected bacterial populations, intestinal fermentation, mucin gene expression and cytokine gene expression in the pig. *British Journal of Nutrition*, 105(5), pp.669–677.

Srinivasa Rao, A. & Umamaheswara Rao, M., 2002. Seasonal growth pattern in

Sargassum polycystum C. Agardh (Phaeophyta, Fucales) occurring at Visakhapatnam, east coast of India. *Indian Journal of Marine Sciences*, 31(1), pp.26–32.

Stallmach, A., Hagel, S. & Bruns, T., 2010. Adverse effects of biologics used for treating IBD. *Best Practice and Research: Clinical Gastroenterology*, 24(2), pp.167–182. Available at: http://dx.doi.org/10.1016/j.bpg.2010.01.002.

Swanson, D., Block, R. & Mousa, S.A., 2012. Omega-3 Fatty Acids EPA and DHA : Health Benefits Throughout Life 1 , 2. *Advances in Nutrition*, 3(1), pp.1–7.

Tabarsa, M. et al., 2012. Fatty acids, amino acids, mineral contents, and proximate composition of some brown seaweeds. *Journal of Phycology*, 48(2), pp.285–292.

Takao, S. et al., 2015. Projecting the impacts of rising seawater temperatures on the distribution of seaweeds around Japan under multiple climate change scenarios. *Ecology and Evolution*, 5(1), pp.213–223.

Takenaka, S. et al., 2001. Feeding dried purple laver (nori) to vitamin B12-deficient rats significantly improves vitamin B12 status. *British Journal of Nutrition*, 85(6), p.699. Available at: http://www.journals.cambridge.org/abstract_S0007114501001210.

Tanoue, T. et al., 2008. In vitro model to estimate gut inflammation using co-cultured Caco-2 and RAW264.7 cells. *Biochemical and Biophysical Research Communications*, 374(3), pp.565–569.

Tedelind, S. et al., 2007. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. *World journal of gastroenterology : WJG*, 13(20), pp.2826–32. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4395634&tool=pmcentrez&rendertype=abstract.

Thahira Banu, A. & Uma Mageswari, S., 2015. Nutritional status and effect of seaweed chocolate on anemic adolescent girls. *Food Science and Human Wellness*, 4(1), pp.28–34. Available at: http://linkinghub.elsevier.com/retrieve/pii/S2213453015000166.

Tiwari, B.K. et al., 2009. Application of natural antimicrobials for food preservation.

Journal of Agricultural and Food Chemistry, 57(14), pp.5987–6000.

Vijayabaskar, P., Vaseela, N. & Thirumaran, G., 2012. Potential antibacterial and antioxidant properties of a sulfated polysaccharide from the brown marine algae Sargassum swartzii. *Chinese Journal of Natural Medicines*, 10(6), pp.421–428. Available at: http://dx.doi.org/10.1016/S1875-5364(12)60082-X.

Walsh, M. & Watson, L., 2011. A market analysis towards the further development of seaweed aquaculture in Ireland. *Irish Sea Fisheries Board, Dublin*, Part 1, pp.1–48. Available at: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:A+Market+Analysis+towards+the+Further+Development+of+Seaweed+Aquaculture+in+Ireland#6.

Wan-Loy, C. & Siew-Moi, P., 2016. Marine Algae as a Potential Source for Anti-Obesity Agents. *Marine Drugs 2016, Vol. 14, Page 222*, 14(12), p.222.

Wang, P. et al., 2014. Anti-metastasis effect of fucoidan from Undaria pinnatifida sporophylls in mouse hepatocarcinoma Hca-F cells. *PLoS ONE*, 9(8), pp.2–9.

Watanabe, F. et al., 1999. Pseudovitamin B12 is the predominant cobamide of an algal health food, spirulina tablets. *Journal of Agricultural and Food Chemistry*, 47(11), pp.4736–4741.

Watanabe1, F. et al., 2002. Characterization and Bioavailability of Vitamin B12-Compounds from Edible Algae. *J Nutr Sci Vitaminoi*, 48, pp.325–331.

Wijesekara, I. et al., 2011. Phlorotannins as bioactive agents from brown algae. *Process Biochemistry*, 46(12), pp.2219–2224.

Wijesinghe, W.A.J.P. et al., 2013. Anti-inflammatory activity of phlorotannin-rich fermented Ecklonia cava processing by-product extract in lipopolysaccharide-stimulated RAW 264.7 macrophages. *Journal of Applied Phycology*, 25(4), pp.1207–1213.

Wijesinghe, W.A.J.P. & Jeon, Y.J., 2012. Biological activities and potential industrial applications of fucose rich sulfated polysaccharides and fucoidans isolated from brown seaweeds: A review. *Carbohydrate Polymers*, 88(1), pp.13–20. Available at: http://dx.doi.org/10.1016/j.carbpol.2011.12.029.

Wilson, K.L. et al., 2015. Effects of increasing water temperatures on survival and growth of ecologically and economically important seaweeds in Atlantic Canada: implications for climate change. *Marine Biology*, 162(12), pp.2431–2444.

Wu, Y. et al., 2014. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *International journal of medical sciences*, 11(11), pp.1185–1200.

Yang, Y., Owyang, C. & Wu, G.D., 2016. East Meets West: The Increasing Incidence of Inflammatory Bowel Disease in Asia as a Paradigm for Environmental Effects on the Pathogenesis of Immune-Mediated Disease. *Gastroenterology*, 151(6), pp.e1–e5. Available at: http://dx.doi.org/10.1053/j.gastro.2016.10.034.

Yin Chia, Y. et al., 2015. Antioxidant and cytotoxic activities of three species of tropical seaweeds. *BMC Complementary and Alternative Medicine*, 15(1). Available at: http://dx.doi.org/10.1186/s12906-015-0867-1.

Zerrifi, S.E.A. et al., 2018. Seaweed bioactive compounds against pathogens and microalgae: Potential uses on pharmacology and harmful algae bloom control. *Marine Drugs*, 16(2).

Zhang, H. et al., 2015. Bioactive dietary peptides and amino acids in inflammatory bowel disease. *Amino Acids*, 47(10), pp.2127–2141.

Zhao, X. et al., 2012. A comparative study of antithrombotic and antiplatelet activities of different fucoidans from Laminaria japonica. *Thrombosis Research*, 129(6), pp.771–778. Available at: http://dx.doi.org/10.1016/j.thromres.2011.07.041.

Zhou, Y. et al., 2016. Increased Enterococcus faecalis infection is associated with clinically active Crohn disease. *Medicine (United States)*, 95(39).

Appendix

Appendix I

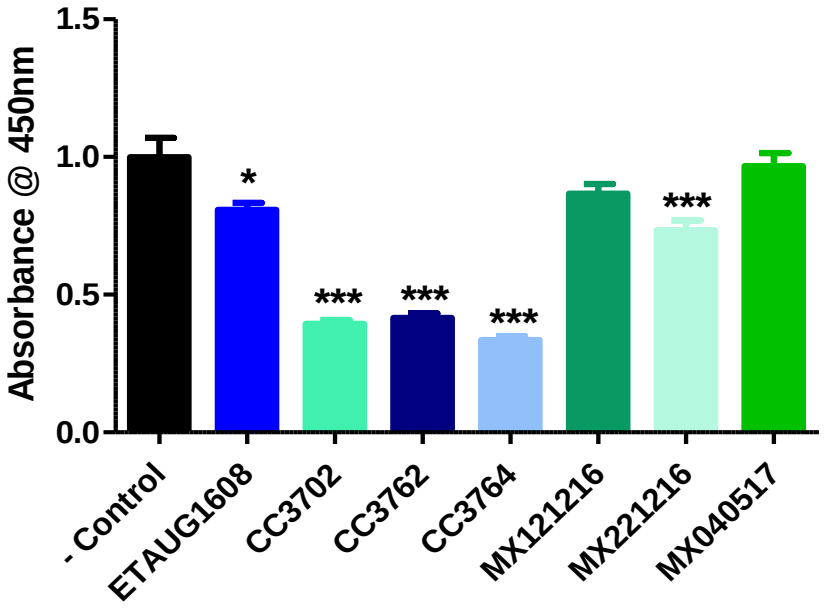


Figure A.1: *Fold change in IL-8 protein expression after 24hr treatment with 25mg/ml of Irish S. latissima (ETAUG1608), Irish A. nodosum (CC3702, CC3762), Scottish A. nodosum (CC3764), Norwegian S. latissima (MX121216), A. esculenta (MX221216) and F. vesiculosus (MX040517). Statistical analysis completed using one-way ANOVA where *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$*

Appendix II

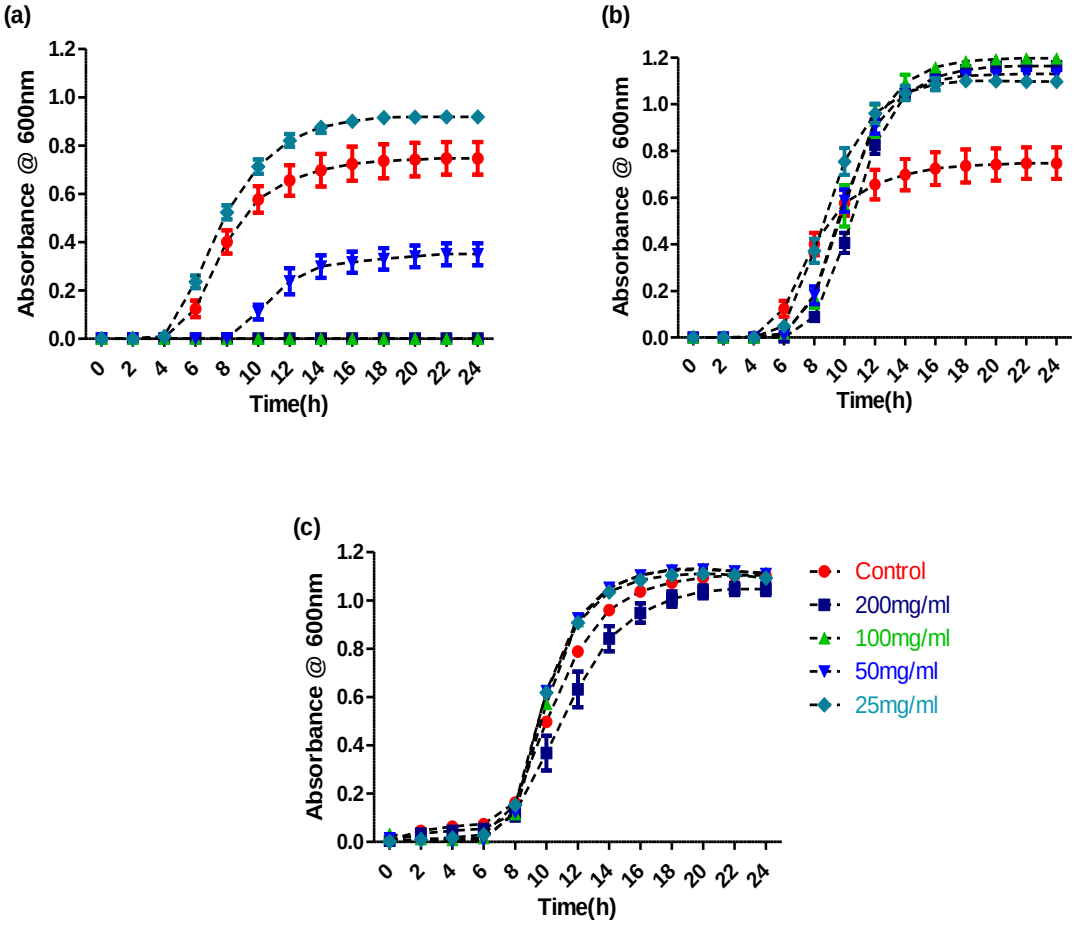


Figure A.2: *Regrowth of Lactobacillus johnsonii after the removal of (a) Norwegian Saccharina latissima (MX121216) (b) Alaria esculenta (MX221216) and (c) Irish Saccharina latissima (ETAUG1608). Growth of L. johnsonii resumed at all concentrations for MX221216 and ETAUG168. Growth of L. johnsonii resumed at 50mg/ml and 25mg/ml for MX121216*