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Ræktun sjávargróðurs - Commercial utilisation of northern Icelandic seaweeds in a closed aqua culturing system

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Dr. Bettina Scholz
Halldór G. Ólafsson
Prof. Dr. Frithjof C. Küpper
Prof. Dr. Hjörleifur Einarsson
Prof. Dr. Ulf Karsten

Report Summary

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Title	*Ræktun sjávargróðurs- Commercial utilisation of northern Icelandic seaweeds in a closed aqua culturing system*	
Authors	*Scholz, B. (Project manager and principal investigator); Ólafsson, H.G. (Field work); Küpper, F.C. (Consultancy); Einarsson, H. (Consultancy); Karsten, U. (Biochemical analysis of utilizable compounds)*	
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Summary:

Seaweeds constitute one of the commercially important renewable marine living resources in Icelandic coastal areas. In the present study overall 16 seaweed species were brought into culture during a course of two years. In detail, two chlorophyceae (*Ulva lactuca*, *Cladophora arcta*), three phaeophyceae (*Fucus spiralis*, *Fucus vesiculosus*, *Dictyosiphon foeniculaceus*) and four rhodophyceae (*Vertebrata lanosa*, *Gracilaria gracilis*, *Odonthalia dentata*, *Palmaria palmata*) were grown over their full life cycle beginning with the isolation of spores. Seven species were only stabilized in culture with minor growth and were therefore not further considered in the present study. Although spore isolation was conducted, the build up of a "seed bank" of Icelandic seaweed strains need far more scientific studies, including the development of species specific methods for the cryopreservation of spores.

The culture of seaweed sporophytes was only realized in 2 L Erlenmeyer flasks and was highly work intensive due to the need to supply the seaweed every week with fresh medium. Also in this aspect further studies including cooperation partners with more suitable facilities such as the University College at Hólar will improve the exploitable biomass and thereby higher the yield of utilizable compounds such as mannitol, laminarin and mycosporine like amino acids (MAA), which were found in higher concentrations in the biochemical analysis of the present study.

Most of the cultivable seaweed species analysed in the present study emerging as good candidates as an alternate source for bioactive substances in pharmaceutical industry and also as a source of food and other health aspects. Particularly, the results of the phytochemical screenings showed the presence of reducing sugars, glycosides, terpenoids, proteins, flavanoids, saponins, tannins, and phenols which might be applicable as pharmaceutical compounds. In addition, the knowledge that the accumulation of utilizable compounds in the seaweeds (e.g. proteins, carbohydrates, lipids) is manipulable using variations in temperature, nutrients, light or salinity gives highly valuable opportunities for future investigations and finally commercial applications.

Keywords: *Seaweed nursery, cultivation, biochemical composition*

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1. Introduction

1.1 The General Biology of Seaweeds

Macroalgae, which are commonly referred to as sea vegetables or seaweed, are classified as Phaeophyta or brown algae, Rhodophyta or red algae, and Chlorophyta or green algae based on the composition of photosynthetic pigments (Hoek et al. 1995). Seaweeds have been used in Iceland for over 1000 years as food, animal fodder, fuel, manure and for dyeing, medicinal purposes, the making of kelp and for various minor uses, and numerous references on this can be found in the extensive literature written in Iceland during the past eight centuries (Hallsson 1964).

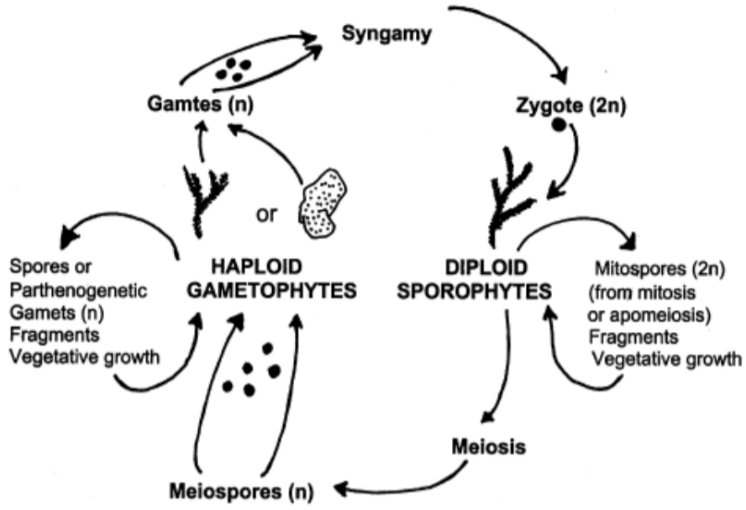

Figure 1. Schematic live cycle of macroalgae (Redmond et al. 2014).

Macroalgae grow primarily in the intertidal zone, and their constant need to protect themselves against oxidative stress from ultra-violet radiation, desiccation and extreme temperature fluctuations at low tide explains the abundance of potent anti-oxidants such as carotenoids, phlorotannins, ascorbic acid (vitamin C), tocopherol (vitamin E), polyphenols, chlorophyll derivatives, and mycosporine-like amino acids (MAAs) in seaweed (Jaspars & Folmer 2013). In comparison with terrestrial plants, algae are particularly rich in iodine, which is essential to the functioning of the thyroid and of the nervous system, in vitamin B12, and in selenium (Jaspars & Folmer 2013). Overall, macroalgae are rich in vitamins, minerals, proteins, poly-unsaturated fatty acids, and dietary fibers, and numerous clinical studies have demonstrated the health benefits of seaweed consumption and linked them to the nutrient composition of seaweed (Shahidi et al. 2008; Venugopal 2011). For example the rhodophyte *Palmaria palmata* is one of the most widely distributed edible seaweeds in Iceland and was an important source of food supply when food variety was scarce in earlier times according to documentaries (Kristjánsson 1980). Nowadays, *P. palmata* is mainly consumed as snacks

and sold in health stores, but there is growing awareness that it should be utilized more as a source of important nutrients and functional ingredients (Plaza et al. 2008). It has been reported that this species has the second highest protein content of all common seaweeds, after *Porphyra tenera* (Nori) (Galland-Irmouli et al. 1999). The essential amino acids (EAAs) can represent between 26 and 50% of the total amino acids (Galland-Irmouli et al. 1999; Morgan et al. 1980). *P. palmata* is also high in iron and many other easily assimilated minerals and trace elements as well as a good source of dietary fibres and vitamins (Morgan et al. 1980; MacArtain et al. 2007). Previous studies have also shown that *P. palmata* contains several classes of hydrophilic antioxidant components including L-ascorbic acid, glutathione (GSH), polyphenols as well as MAAs (Yuan et al. 2005; 2009)

In general, the life cycles of macroalgae are complex and diverse, with different species displaying variations of annual and perennial life histories, combinations of sexual and asexual reproductive strategies, and alternation of generations (Fig. 1). Each species has its characteristic life history strategy, which must be understood and brought under control in order to develop appropriate cultivation techniques. The successful cultivation of commercially important seaweeds such as *Laminaria* and *Porphyra* in Asia was possible after life cycles were first understood, and incorporated into protocols to produce seed in nursery operations (Tseng, 1987; Choi et al., 2002).

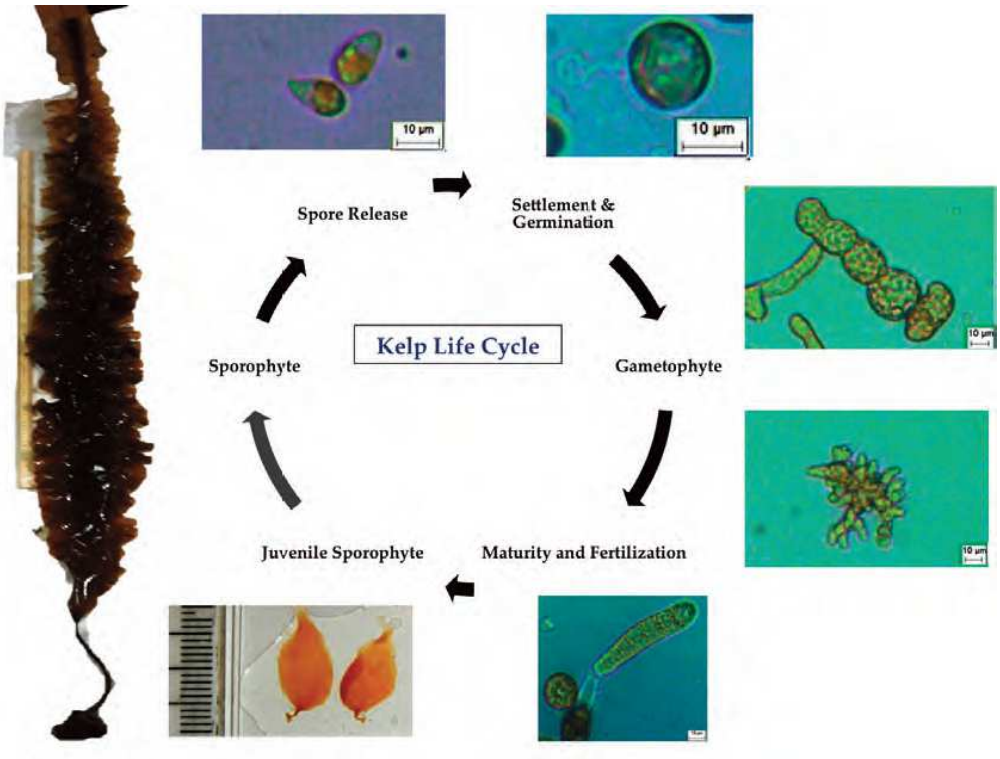

Figure 2. Kelb live history stages (Redmond et al. 2014).

Many seaweeds have a biphasic or what is called a haploid-diploid life cycle, and these species alternate between haploid (having a single set of chromosomes in the nucleus, designated by the letter 'n') and diploid (having two sets of chromosomes in the nucleus, designated by '2n'). Diploids produce haploid spores by meiosis, a set of two special divisions of the cell nucleus. These spores

develop into male and female aploid adults called gametophytes (e.g. Kelb in Fig. 2, Lüning 1990). The adult gametophytes produce eggs and sperm which can unite to grow into diploid adults, the sporophytes, completing the life cycle. This is the generalized life cycle of seaweeds. There are some complexities. Usually, male and female gametophytes are dioecious, that is the sexes are in separate individuals, and there are male 'plants' and female 'plants'. Some species, though, are monoecious, both sexes being housed in the same individual 'plant'. Normally, gametes (eggs and sperm) are released into the water where the egg is fertilized and forms a diploid zygote which settles to the bottom, attaches to the substrate, and grows into a mature alga. However, red algae have a modified biphasic cycle – actually a triphasic cycle - with the addition of a short-lived carposporophyte, a diploid stage formed on the surface of the female gametophyte thallus by the union of haploid gametes (Thornber 2006). This carposporophyte lives on the female thallus from which it acquires nutrients. It produces spores, the diploid carpospores, which are released into the water, settle, and grow into mature, free-living tetrasporophytes. The tetrasporophytes, by meiosis, produce tetraspores (each spore is formed in a group of four) that are released into the water column where they settle to become male and female gametophytes. Another complication is that mature free-living phases of some species have morphologies that are heteromorphic with independent stages that are distinctly different from each other, and other species are isomorphic with phases that look almost identical to each other. Those algae with isomorphic phases sometimes have different demographics; they are differently distributed as gametophyte or sporophyte populations, depending on seasons, the amount of shelter or wave exposure, and other environmental factors (Lüning 1990).

1.2 Seaweed Compounds and their Commercial Applications

Seaweeds are known for their richness in polysaccharides, minerals and certain vitamins (Arasaki & Arasaki 1983), but they also contain bioactive substances like polysaccharides, proteins, lipids and polyphenols, with antibacterial, antiviral and antifungal properties, as well as many others both *in vitro* and *in vivo* (Kumar et al. 2008; Audibert et al. 2010; Li et al. 2011; Thomas & Kim 2011; Wang et al. 2012; Lopes et al. 2012). This gives seaweed great potential as a supplement in functional food or for the extraction of compounds. These compounds are in most cases storage compound and therefore highly class specific.

For example, the storage polysaccharide of brown algae is laminarin, a vacuolar b-1,3-glucan with occasional b-1,6-linked branches (Percival & Ross 1951). This polysaccharide is polydisperse, consisting of a minor G-series with polymers containing only glucose residues, and a more abundant M-series with glucans terminated with a 1-linked d-mannitol residue (Michel et al. 2010). This polysaccharide is polydisperse, consisting of a minor G-series with polymers containing only glucose residues, and a more abundant M-series with glucans terminated with a 1-linked d-mannitol residue (Read et al. 1996). Experiments with radioactive C demonstrated that laminarin and mannitol are interchangeable storage compounds in phaeophytes, as are sucrose and starch in higher plants (Yamaguchi et al. 1966) or saccharose in Chlorophyceae. In kelps, mannitol can be remobilized and translocated via the sieve tubes from mature tissues to supply the rapidly growing parts of the alga with C (Schmitz & Lobban 1976; Lobban & Harrison 1994). In contrast, the heteroside floridoside (a-D-galactopyranosyl-[1–2]-glycerol) is considered to be the main photosynthetic and reserve product

in all orders of the Rhodophyta except the Ceramiales. In general, members of the Ceramiales synthesize and accumulate the chemically related digeneaside (a-D-mannopyranosyl-[1–2]-glycerate) (Karsten et al. 1999, Fig. 3).

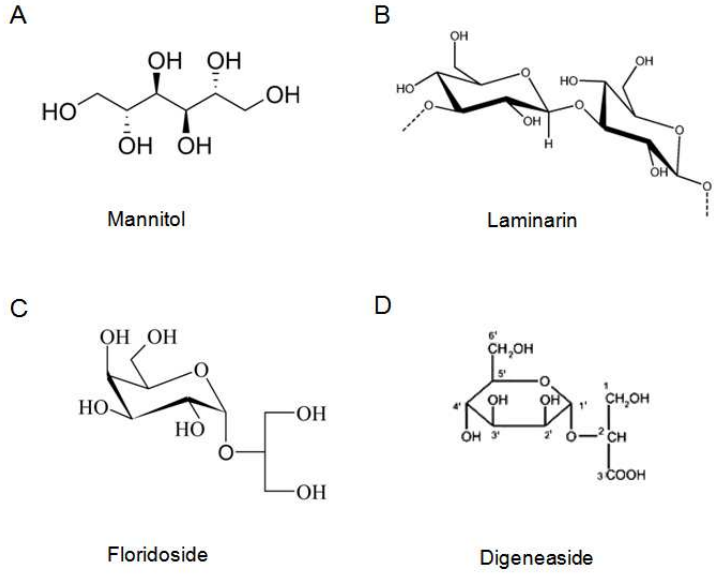

Figure 3. Chemical structures of seaweed storage compounds. A) and B) are found in brown seaweeds, whereas C) and D) are accumulated in red seaweeds.

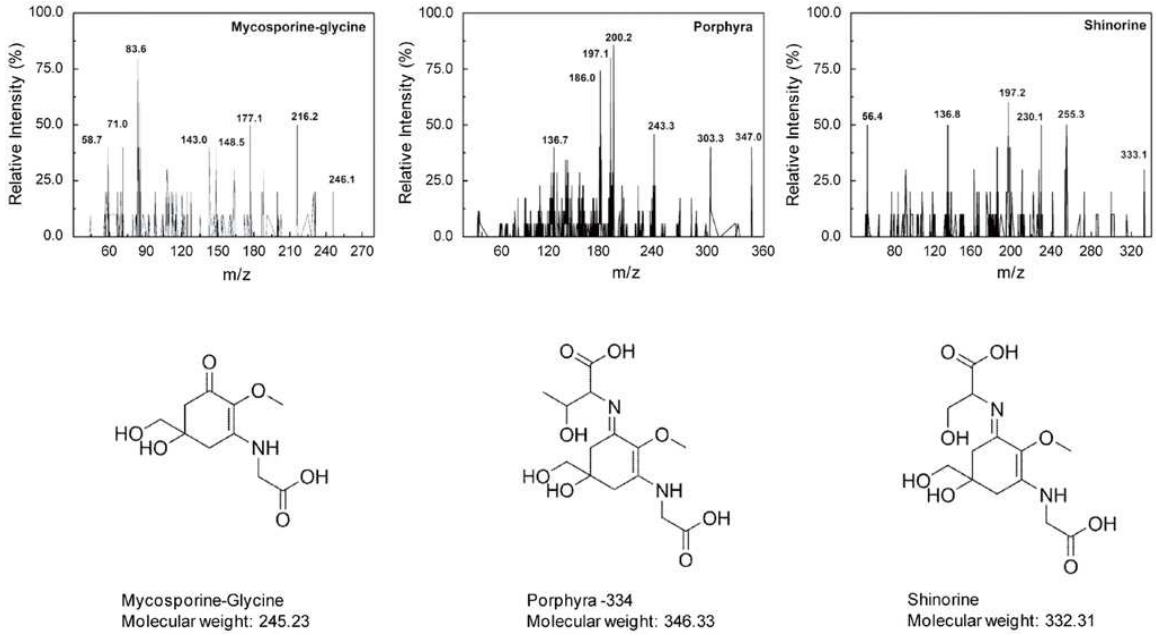

Figure 4. MS/MS analysis of mycosporine-Gly, shinorine, and porphyra-334 from *Chlamydomonas hedleyi* (Suh et al. 2014)

Other bioactive compounds originating from the need of the seaweeds to cope to environmental conditions such as high ultraviolet radiation (UVR). For algae exposed to enhanced UVR for substantial parts of their life-cycles, strategies that passively screen this waveband will contribute to prevent UV-induced damage to essential bio molecules such as DNA and proteins (Bischof et al. 2006; Karsten et al. 2009). In addition, UV-screening may also save metabolic energy by reducing the need for constantly active avoidance and repair processes. The most common photo protective sunscreens in many, but not all algal taxa and cyanobacteria are the MAAs, a suite of chemically closely related, colourless, water-soluble, polar and at cellular pH uncharged or zwitterionic amino acid derivatives. MAAs are related to fungal mycosporines which were first isolated from sporulating mycelia (Karsten et al. 2009), and consist of aminocyclohexenone or aminocyclohexenimine ring systems. The various MAA structures result from N-substitutions of different amino acid moieties to the cyclohexenone or cyclohexenimine chromophore. At present, there are only few known aminocyclohexenonederived MAAs such as mycosporine-glycine, which typically exhibit their absorption maximum in the UVB (280–315 nm) range. All others, about 20 described MAAs are derivates of the aminocyclohexenimine structure which maximally absorbs at UVA (315–400 nm) wavelengths (Cockell & Knowland 1999; Karentz 2001; Shick & Dunlap 2002; Karsten et al. 2009) (Fig. 4).

Sunscreen use has significantly expanded in the last decades as consequence of the perception that sun exposure may the main cause for the development of skin cancer and the photo aging process (Maier & Korting 2005). Further reports have shown that, in the last 20 years, the incidence of non-melanoma skin cancer (NMSC) has increased significantly (Halpern & Kopp 2005). Daily application of sunscreen products is highly recommended by health care professionals and it has been suggested that the incidence of NMSC can be drastically reduced (or even prevented) by avoidance of excessive exposure to UV radiation and by using sunscreen (Halpern & Kopp 2005).

Recent researches have given the evidences of the harmful effects (radicals' generation, risks of skin cancer and estrogen like-effects) of the available chemical sunscreens in the market and their regular application to the skin (Bhatia et al. 2011). These chemical agents were proven as primary causative agents for increasing the cancer risk by virtue of their abundant free radical generating properties and estrogen-like effects. These effects are similar to many banned chemicals such as dichloro-diphenyl trichloroethane (DDT), dioxin, and polychlorinated biphenyls (PCBs), but they are still present in the market due to profit gaining purpose of the chemical industries. Most of the chemical sunscreens contain 2–5% of UVA and UVB blockers as the active ingredients. Most of these UV-blocker compounds are cancer causing elements, e.g. Benzophenone (and similar compounds) and Avobenzone (powerful free radical generators), Padimate-O or other *p*-aminobenzoic acid (PABA) derivatives (DNA damaging effects), and Triethanolamine (formation of cancer causing nitrosamines). Most of the sunscreen compounds are either UVA or UVB protective and are usually combined with other sunscreen chemicals to produce a "broad-spectrum" product. Furthermore, in sunlight, some show instability after a particular period of time. It clearly indicates the need of a potent, stable and broad-spectrum group of agents which are devoid of their hazardous effects to the skin. Here, mycosporine like amino acids stand as a good example due to their excellent antioxidant and sun protection activities without causing any harmful effects (Bhatia et al. 2011). Until now only one product called Helioguard® 365 that contains mycosporine-like amino acids from the red macroalga *Porphyra umbilicalis* has been commercialized (Llewellyn & Airs 2010).

The traditional use of seaweed as food has been traced back to the fourth century in Japan and the sixth century in China. Today those two countries and the Republic of Korea are the largest consumers of seaweed as food (McHugh 2003). Whilst food has long been used to improve health, our knowledge of the relationship between food components and health is now being used to improve food. Although most foods can be considered "functional", in the context to seaweed utilization, the term is reserved for foods and food components that have been demonstrated to provide specific health benefits beyond basic nutrition. Widespread interest in select foods that might promote health has resulted in the use of the term "functional foods". Food and nutrition science has moved from identifying and correcting nutritional deficiencies to designing foods that promote optimal health and reduce the risk of disease. Today's science and technology can be used to provide many additional functional foods, and future scientific and technological advances promise an even greater range of health benefits for consumers. Functional foods can provide health benefits by reducing the risk of chronic diseases and enhancing the ability to manage chronic diseases, thus improving the quality of life (e.g. Løvstad Holdt & Kraan 2011).

Figure 5. Products based on seaweed supplements.

Various red and brown seaweeds are used to produce food additives such as agar, alginate and carrageenan (hydrocolloids). Seaweed meal, used an additive to animal feed, has been produced in Norway, where its production was pioneered in the 1960s. Other seaweed products comprise fertilizers and cosmetic products, such as creams and lotions (Fig. 5).

Over the last twenty years there have been some large projects that investigated the possible use of seaweeds as an indirect source of fuel. The idea was to grow large quantities of seaweed in the ocean and then ferment this biomass to generate methane gas for use as a fuel. The results showed

the need for more research and development, that it is a long-term project and is not economic at present. In addition there are potential uses for seaweed in wastewater treatment. Some seaweeds are able to absorb heavy metal ions such as zinc and cadmium from polluted water. The effluent water from fish farms usually contains high levels of waste that can cause problems to other aquatic life in adjacent waters. Seaweeds can often use much of this waste material as nutrient, so trials have been undertaken to farm seaweed in areas adjacent to fish farms.

In the last three decades the discovery of metabolites with biological activities from macroalgae has increased significantly. However, despite the intense research effort by academic and corporate institutions, very few products with real potential have been identified or developed. Substances that currently receive most attention from pharmaceutical companies for use in drug development, or from researchers in the field of medicine-related research include: sulphated polysaccharides as antiviral substances, halogenated furanones from *Delisea pulchra* as antifouling compounds, and kahalalide F from a species of *Bryopsis* as a possible treatment of lung cancer, tumours and AIDS. Other substances such as macroalgal lectins, fucoidans, kainoids and aplysiatoxins are routinely used in biomedical research and a multitude of other substances have known biological activities (e.g. Redmond et al. 2014).

1.3 Tank based Indoor Seaweed Culturing Systems

Figure 6. Examples of seaweed indoor cultivation systems.

Whole seaweeds have been incorporated into a range of foods including meat, and bakery products. While utilizing the whole seaweed thallus could be useful in tackling dietary iodine insufficiency, consumption of some species and sources of seaweed has also been associated with risks, such as

toxicity from high iodine levels, or accumulation of arsenic, heavy metals and contaminants (Bouga & Combet 2015). The concerns over toxicity and exposure to high level of iodine are mitigated by scrutiny of the seaweed species (there are more than 50 commonly eaten species) and the waters in which it was farmed or harvested. For example, the iodine content of seaweed varies from 16 µg/g in some Nori species (*Porphyra tenera*) to 8165 µg/g in Icelandic *Laminaria digitata* (Teas et al. 2004). It has been shown that increased iodine concentrations in seaweeds are directly related to environmental stress such as high ultraviolet radiation (Küpper et al. 2008; Medrano-Macías et al. 2016). Thus indoor tank based culturing systems have clearly the advantage to provide controlled environmental conditions. Some examples of such indoor culturing systems are illustrated in Fig. 6.

Although wild harvest supports a significant portion of seaweed industry, there is an ever increasing amount of seaweed production from aquaculture, principally in Asia and South America (Chile). Seaweed aquaculture makes up a significant portion of organisms cultured worldwide (~19 million metric tons) with a value of ~US$5.65 billion (FAO 2012). Aquaculture production is dominated by kelps (*Saccharina japonica* and *Undaria pinnatifida*), tropical red algal species ("carrageenophytes" species including *Kappaphycus* and *Eucheuma*), nori (including *Porphyra* and *Pyropia* species), and the red algal agarophyte species known as *Gracilaria*. China is the world's top producer of cultured seaweeds, though other countries in Asia (Japan, Korea, and the Philippines) and in Europe (France, Ireland, Norway, Scotland, and Spain) also grow seaweed. In North America, the seaweed industry is comprised of small wild harvest cottage operations located along the East and West Coasts of Canada and the United States. Recent development in culture technologies, however, have led to the development of a small sugar kelp industry in the Northeast. Today, most of the commercial harvest of macroalgae for food and other products comes from cultivated algae derived from hatchery propagated seed stock, rather than from harvest of natural stands (Redmond et al. 2014). Although wild harvest supports a significant portion of seaweed industry, there is an increasing amount of seaweed production from aquaculture, principally in Asia and South America (Chile). As populations expand, culture of seaweed will be important to supplement the wild resource. Seaweeds can be cultivated in the sea on suspended lines, rafts, or nets, or on land in tank based culture systems (Fig. 3). The development of a seaweed aquaculture industry can also encourage development of other aqua cultured species that are higher up in the food chain. Seaweeds are bio extractive organisms, taking up excess nutrients generated by other species, such as fish or shrimp. The integrated culture of fed aquaculture (fish and shrimp) with extractive aquaculture (seaweed and shellfish) is called 'Integrated Multi Trophic Aquaculture', or IMTA (Neori et al. 2007).

In order to grow seaweed in an closed aqua culturing system, it is necessary to have a source of young plants. In Asia, native species have been isolated from wild populations; strains have been selected for desirable traits and are maintained as "seed" cultures. Seaweed farmers often belong to a cooperative and obtain "seed" nets or lines from a seaweed culture facility (i.e. a seaweed nursery, Redmond et al. 2014).

2. Objectives and Aims of the Study

The main objective is to grow and screen macroalgae, isolated from northern Icelandic coastal habitats, for their potential as new sources of pharmaceuticals and food additives. The project includes the build-up of a suitable culturing system and extension of the culture collection of algal strains at BioPol in order to serve aqua cultures as well as science and applied institutes worldwide with Icelandic taxa. Using the Hunaflói (NW, Iceland) as study site, we propose to conduct a study providing in its final consequence novel compounds for the Icelandic health and aquaculture industries.

Therefore, the aims of the present study were:

1. to set up a culture collection of Icelandic macroalgae in form of their spores (spore or seed nursery) as a resource for both academic and applied users;

2. to optimise growth conditions of selected species as intermediary step for the upscale;

3. to establish a tank based closed culture system for the sporophytes, in order to obtain biomasses for further studies;

4. to investigate biomass extracts of the adult macroalgae using a combination of phytochemical and biological activity screening;

5. to further describe selected strains on basis of their biochemical composition.

3. Material and Methods

3.1 Collection of Wild Material

Two sites, located in the north-western part of Iceland, were used for the collection of wild seaweed material (Fig. 7). The first side is a steep coast characterised by cliffs and rock pools (Figs. 8A, B), whereas the second one is a tidal flat in which stones functioning as a sand trap (Figs. 8C, D). Seaweeds were sampled during low tide from February until October 2015 and March until June 2016. Only healthy and strong coloured plants were collected, targeting mainly individuals of the genera *Palmaria, Ulva, Fucus, Gracillaria.* Initially whole plants from different species were collected and trials were started to bring them into culture, using artificial seawater salt (Tropic Marin) enriched with Provasoli (cf. section 3.3). To obtain spores for seeding, reproductive sorus tissue from wild plants were carefully collected and processed in the laboratory. In the case of Kelp (e.g. *Saccharina latissima;* Phaeophyceae; order Laminariales) reproductive plants can be found throughout the year, though the primary reproductive periods occur in the spring and autumn months (Egan & Yarish 1990; van Patten 1992). In the case of *Gracillaria* (Phylum Rhodophyta) only whole plants were collected and reprocessed as described in section 3.2.

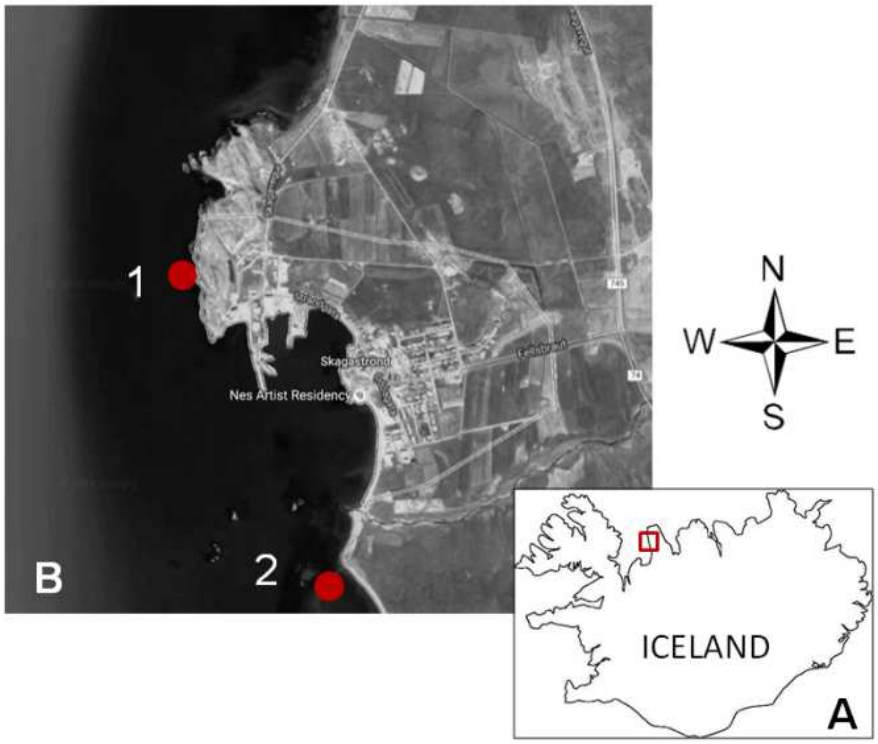

Figure 7. Sampling locations in the north-western part of Iceland neat Skagaströnd. A) Survey map; B) Sampling sites (1. rock pools, 2. tidal flat behind the stream)

Figure 8. Seaweeds at the two different sampling locations A, B) rock pool, C,D) tidal flat.

3.2 Spore and Tip Isolation

Overall three methods were used for the spore isolation of the different seaweed taxa. In the case of species of the order Laminariales (e.g. *Saccharina latissima*) the most important step in culture was the cleaning process of the collected material. Contaminated material (e.g. protozoans, fungi, other micro- and macroorganisms, or cyanobacteria) were found to overgrow the seaweeds in between weeks and finally led to the death of the plant. The cleaning process involved a series of rinses and wipes in order to remove microscopic contaminants on the surface of the blades. Sorus tissue was excised from all non-reproductive tissue, and cut into manageable strips. Both sides were carefully and gently scraped with the edge of a sterile scalpel. This was followed by a series of rinsing and wiping down of the blades in a shallow tray and wiping with sterile paper towels, including a final rinse on both sides with a squirt bottle. After cleaning, the sorus tissue was placed between damp sterile paper towels and refrigerated overnight at 10°C in an autoclaved plastic bag. This step allowed

the sorus tissue to undergo a gentle desiccation period in order to stimulate spore release with re-immersion in artificial seawater (Tropic Marin®, Aquarientechnik, Wartenberg, Germany; Fig. 9).

Figure 9. Sorus tissue preparation and spore release (Redmond et al. 2014).

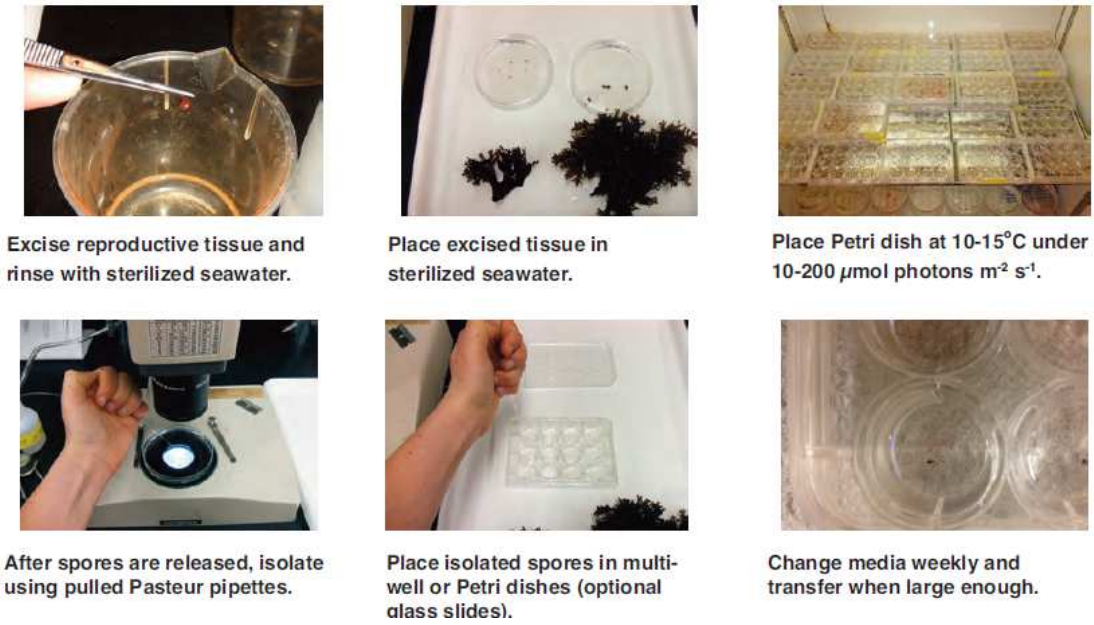

Figure 10. Spore isolation procedure demonstrated for *Chondrus crispus* (Redmond et al. 2014).

The second method for spore isolation, which was used for *Dictyosiphon foeniculaceus, Vertebrata lanosa, Fucus, Cladophora, Odonthalia* and *Ulva* included the use of autoclaved Pasteur pipettes

which were heated above a flame and pulled to a fine tip. This fine tip allowed individual spores to be picked up by using vacuum pressure created by placing the thumb over the end of the pipette to seal it, locate an individual spore under the dissecting microscope, carefully place the pipette adjacent to the spore and slowly release the thumb from the end of the pipette to allow the spore to be collected in the pipette (Fig. 10). The spores were transferred into a single well of a 96 well culture plate with approximately 1 of sterile Provasoli´s. The third method was used for *Palmaria*. During this procedure individual spores were isolated by the transfer of the spores into sterile shallow Petri dishes containing pieces of sterilized glass microscope slides. The spores settled onto the glass surface to grow, allowing an ease of transfer and manipulation.

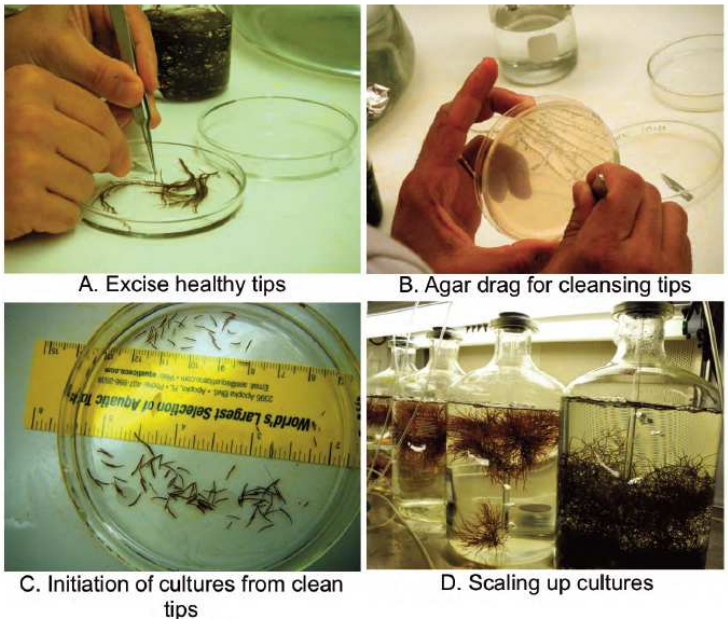

Figure 11. Isolating and cleaning tips for establishing clean cultures (Redmond et al. 2014).

In the case of *Gracillaria*, clean, healthy (deep pigmentation), actively growing, and/or reproductive 'parent' fronds (sometimes referred to a thallus) were selected from the collected wild material in the laboratory under sterile conditions. Fronds were chosen initially from wild populations and later from existing cultures. To obtain organisms for culture initiation, tips were cut from the parent frond, cleaned thoroughly, and placed in favourable growing conditions. Once a number of healthy tips were excised, each tip was individually cleaned in sterile seawater. This was followed by an drag through prepared agar plates (5 $g \cdot L^{-1}$ agar in distilled water with 35 $g \cdot L^{-1}$ artificial seawater salt). The procedure was repeated three times (Fig. 11).

3.3 Cultivation of Gameto- and Sporophytes

The three most important components of a culture system are seawater media (seawater and nutrients), temperature and light. In most cases the sporulation is close related to optimum temperature and light conditions. These optima are highly group specific and vary with geographical

area. In numerous trials were optimum cultivation parameter tested for gameto- and sporophytes of different northern Icelandic representatives of phaeo-, rhodo- and chlorophyceae (Table 1). While the cultivation of sporophytes was conducted in 2 L Erlenmeyer flasks (Fig. 12), gametophytes were first grown in sterile Petri disks and later transferred into 50 and 100 mL flasks. Cultures were supplied in weekly intervals with fresh medium. Overall three different media were tested: Provasoli´s, f/2 and Von Stosch Enriched Seawater (media receipts are given in the Appendix). In addition, Provasoli´s was tested in three different nutrient concentrations (0.25, 0.5 and 0.75 %). The artificial seawater salt (Tropic Marin®) was dissolved in de-ionized water and was used with a salinity of 30 and 32, respectively, and pH of 8.2. The salinities are according to the Practical Salinity Scale (PSU). For the light intensities full spectra daylight bulbs (Phillips, Germany, Master TL-D 18W/840) were used. Conductivity, temperature, dissolved oxygen and pH were measured using handheld probes (YK-31SA, YK-200PATC, YK-200PCD and YK-2001PH, SI Model 33, Engineered Systems and Designs-Model 600, Philips W9424). Photosynthetic active radiation (PAR, 400–700 nm) was measured with an underwater spherical quantum sensor LI-193SA connected to a Licor Data Logger LI-250A. For diatom control: 0.2 % (v/v) germanium dioxide was used.

Table 1. Optimum culture parameter for gametophyte and sporophyte culture systems adapted to northern Icelandic climate conditions.

Culture Conditions	**Gametophyte Phase**		**Sporophyte Phase**	**Additional Remarks**
	Release and settlement of Spores	Sporophyte formation	Juvenile Sporo-phytes	
Temperature [°C]	5-8	8-10	8-12	
Light [µmol photons m^{-1} s^{-1}]				
Phaeophyceae	200	500	500	Use of full spectra day light bulbs
Rhodophyceae	30	30	50	
Chlorophyceae	25	30	30	
Photoperiod (light:dark)				
Phaeophyceae	8:16	8:16	8:16	Use of timer
Rhodophyceae	8:16	12:12	12:12	
Chlorophyceae	8:16	12:12	12:12	
Salinity	30	30	32	
Seawater	artificial	artificial	artificial	Use of Tropic Marin as artificial seawater salt
Nutrient Media				
Phaeophyceae	PES 0.5 strength	PES 0.75 strength	PES 0.75 strength	PES = Provasoli´s Enriched Seawater medium
Rhodophyceae	PES 0.25 strength	PES 0.5 strength	PES 0.75 strength	
Chlorophyceae	PES 0.5 strength	PES 0.5 strength	PES 0.75 strength	
Aeration level				
Phaeophyceae	low	medium	medium	Cellulose stoppers and sterile 10 mL pipettes were used for aeration with a pump
Rhodophyceae	low	medium	strong	
Chlorophyceae	low	medium	medium	

Figure 12. Examples of sporophyte cultivation of different seaweed taxa in the climate chamber at BioPol ehf. laboratory. A) shelves with seaweed cultures in 2 L Erlenemeyer flasks; B) media test series with *Ulva lactuca* (chlorophyceae); C) *Ulva lactuca* after 3 months cultivation; D)-H) cultivation of different rhodo- and phaeophyceae.

3.4 Preservation and Identification of Seaweeds

For preservation and identification of seaweeds, specimens were cleaned of sand particles and other adhering materials such as epiphytes. Seaweeds were washed in a tray containing fresh water (half filled) and were spread on paper sheets with the help of a brush in order to minimize the overlapping of the specimen. In the next step, the sheets were removed and the seaweeds were properly arranged using forceps or needles if required. To blot dry, sheets were placed on carton sheets to remove the remaining water from the herbarium. Then a plastic cloth was placed on the top of the individual seaweed in such a way that it covered the entire specimen. After that another sheet was placed over the herbarium sheet. Once, all the seaweeds were prepared, the herbaria were piled one

above the other and then placed into a wooden press. After 24 h drying at room temperature the papers were replaced. This process was repeated until the seaweeds were free of moisture. This method still allows to extract DNA even after preservation. Pictures of the individual species cultured during this project are given in the Appendix.

The following literature was used for identification of the taxa: Brodie (2003); Dixon (2011); Fletcher (2011); Irvine (2011a, b); Burrows (2013); Christensen (2013) and Maggs (2013).

3.5 Determination of Growth

Growth rate was calculated for each species (replicated) by the increase in wet weight and presented as percentage growth per week using the formula of Penniman et al. (1986):

$$SGR = \% [(Gt/Go)1/t - 1] \times 100$$

SGR = specific growth rate (% in wet weight per week),
Gt = weight after t weeks,
Go = initial weight, t = time in weeks

3.6 Biochemical Analysis of Cultivable Seaweeds

3.6.1 Gross Compositions

Seaweeds (sporophytes) were harvested from the 2 L Erlenmeyer flasks, frozen and freeze dried (=lyophilisation) before analysis. Total protein, lipid, carbohydrate, and ash contents of the species were determined on seaweeds growing under the standardised conditions (12 °C, 32 PSU, 12:12 light:dark cycle; PES 0.75 strength; cf. Table 1). All tests were performed in triplicate.

Proteins

Protein was determined spectrophotometrically using a Pierce test kit (BCA 23225) based on the procedure of Lowry et al. (1951) and standardised bovine serum albumin (BSA). In brief, lyophilized seaweeds were prepared for analysis in four steps, with cooling intervals in an ice bath. Firstly, the filters were suspended in 4 ml 0.1 M NaOH in a 15 ml polypropylene test tube. Then the filter was ground in a tissue homogeniser for 1 min and sonicated 20 s. Samples were diluted to 8 ml with 0.1 M NaOH, heated to 60 °C, sonicated for 10 s and centrifuged (4 °C, 800 g, 10 min). A total of 10 µl of each supernatant was transferred to each triplicate well in a 96 well microplate. 200 µl of BCA reagent was added to each well and incubated for 30 min at 37 °C. Finally, the microplate was analysed at 526 nm with a microplate reader.

Carbohydrates

Total soluble carbohydrates were determined using the phenol sulphuric acid method of Kochert (1978) and Ben-Amotz et al. (1985) incorporating the modifications of Mercz (1994) and Buttery (2000). The samples were homogenized in 1 M H_2SO_4 and after heating at 100 °C for 60 min, 0.3 ml of the supernatant was transferred into a fresh vial and made up to 2 ml with deionized water. Sets of glucose standards were prepared and 1 ml of 5 % (w/v) phenol solution was added to all samples, after which, 5 ml of concentrated H_2SO_4 was added. Absorbance was read at 485 nm.

Lipids

The total lipid determination was based on the method of Bligh and Dyer (1959) as modified by Kates and Volcani (1966) and adapted by Mercz (1994) and Buttery (2000) using methanol: chloroform: deionised water (2:1:0.8, v/v/v). The extracts were dried under a stream of ultra-pure N_2 gas and placed in a vacuum desiccator over silica gel overnight and then weighed. The lipids were resuspended in 100 µl 2:1 (v/v) chloroform:methanol, sealed, flushed with N_2, and then stored at –80 °C until lipid classes analysis. Lipid class were quantified by thin layer chromatography-flame ionisation detection using a Lactroscan Analyzer with methods described by Kreeger et al. (1997).

Ash content

Total ash weights were determined by following the method of Buttery (2000). The glass fibre filters were placed within crucibles before combustion at 475 °C for 24 h. After combustion, the samples were weighed to five decimal places and total ash was calculated by subtracting the initial weight of the filter.

3.6.2 Utilizable Compounds

The analysis of utilizable compounds and secondary metabolites of the different seaweed groups were conducted as comprised in Table 2.

Table 2. Overview of the conducted analysis of northern Icelandic sporophytes grown at BioPol ehf. laboratory.

Phylum	CN	Mannitol/ Laminarin	Floridoside / Digeneaside	MAAs	Phytochemical Screenings (cf. 6.3.3)
Phaeophyceae	++	++			++
Rhodophyceae	+		+	+	+
Chlorophyceae	+				+

++ the plants were subdivided as far as possible in leaves and stems before freezing

+ the whole thallus was used for analysis

Abbreviations: CN: Carbon and Nitrogen analysis; MAAs: Mycosporine-like amino acids

Carbon and Nitrogen (CN)

For analyzing CN contents according to the method described by Graiff et al. (2015), freeze-dried algal material was ground to powder using a ball mill, and three subsamples of 2 mg from each algal thallus were loaded and packed into tin cartridges (6 × 6 × 12 mm). These packages were combusted

at 950 °C and the absolute contents of C and N were automatically quantified in an elemental analyzer using acetanilide as standard.

Mannitol and Laminarin

Mannitol was extracted from freeze-dried subsamples of 10–20 mg powdered algal material and quantified, following the HPLC method described by Karsten et al. (1991).
Laminarin was identified and quantified according to the method described by Graiff et al. (2016), using a cold water extraction method coupled to a new quantitative liquid chromatography-mass spectrometrical method (LC-MS).

Floridoside and Digeneaside

Compounds were quantified by 13C-nuclear magnetic resonance spectroscopy (13C-NMR) according to the method described in Karsten et al. (1999). Briefly, 100 to 250 mg of algal dry weight (DW) was extracted in 5 mL of 70% ethanol (v/v) for 3 h in a water bath at 708 C. After centrifugation at 5000 x *g,* the supernatant was evaporated under vacuum to dryness and re-dissolved in 0.5 mL of D_2O (99.98%) for NMR spectroscopy. The 13C NMR spectra were recorded on a Bruker AM-500 spectrometer at 125.77 MHz. Floridoside were verified by comparing with standards isolated from red algae (Karsten et al. 1993). Digeneaside was identified using a standard isolated from the red alga *Ceramium flaccidum* (Kützing) Ardisonne.

Mycosporine-like* amino acids *(MAAs)

MAAs were analysed according to the method of Karsten et al. (2009). Briefly, three replicate macroalgal samples were extracted for 1.5–2 h in screw-capped centrifuge vials filled with 1 mL 25% aqueous methanol (v/v) and incubated in a water bath at 45°C. After centrifugation at 5,000 g for 5 min, 700 L of the supernatants were evaporated to dryness under vacuum. Dried extracts were re-dissolved in either 700 L 100% methanol, distilled water or the HPLC eluent [2.5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water]. After centrifugation at 5,000 g for 5 min the extracts were passed through a 0.2 µm membrane, and afterwards analyzed with an Agilent HPLC system. MAAs in all extracts were one after another separated on three different stainless-steel HPLC columns. The mobile phase was always 2.5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water. MAAs were detected online with a photodiode array detector at 330 nm, and absorption spectra (290–400 nm) were recorded each second directly on the HPLC-separated peaks. Identification was done by spectra, retention time and by co-chromatography with pure standards of palythine, porphyra-334 and shinorine. Quantification was made using the molar extinction coefficients given in Karsten et al. (1998).

3.6.3 Screening for further utilizable Compound Classes

For the phytochemical screening, the lyophilisated biomass of each species was weighted, equalized to a final weight of 3 g fresh weight and sonicated 3 times for 2 min under cooling conditions (Branson 2800, Emerson Electric Co, Ferguson, Missouri, United States). The homogenized biomass was then extracted in three different solvents (each 1 h) in an all-glass filtration chamber, using 50 mL ethanol (99.5 %; for flavonoids, alkaloids, phenols, tannins, saponins, etc), 50 mL methanol (80 %; for aldehydes and sterols) and 50 mL *n*-hexane (PUFAs), following different methods referenced in

Table 3. With the exception of the sonication, the same extraction procedure was conducted for the evaporated supernatant extracts. The residues from all extractions were evaporated and re-dissolved in 100 ml aqueous EtOH (40%), filtered (Whatman GF/C, 47 mm), and used for the phytochemical screening.

Table 3. Methods used during the phytochemical screenings.

Compound group	**Extraction Solvent**	**Calibration standard**	**Method**	**Ref.**
Aldehydes	MeOH (80%)	Formaldehyde CH_2O	Schiff's and Fehling's tests*	Turner (1916)
Alkaloids	EtOH (99%)	Piperine $C_{17}H_{19}NO_3$	Mayer´s and Wagner`s reagent*	Scholz & Liebezeit (2006)
Carbohydrates	EtOH (99%)	D-glucose $C_6H_{12}O_6$	Fehling's Test	Tiwari et al. (2011)
PUFA	*n*-hexane	Stearidonic acid $C_{18}H_{28}O_2$	Argentation thin layer chromatography[2]	Wilson & Sargent (1992)
Flavonoids	EtOH (99%)	Quercetin $C_{15}H_{10}O_7$	Alkaline Reagent Test	Tiwari et al. (2011)
Glycosides	EtOH (99%)	Oleandrin $C_{32}H_{48}O_9$	Keller-Killiani Test	Usunobun et al. (2015)
Phenols	EtOH (99%)	Hydroquinone $C_6H_6O_2$	Folin-Ciocalteu reagent/ $FeCl_3$	LeBlanc et al. (2009)
Phytosterols	MeOH (80%)	Ergosterol $C_{28}H_{44}O$	Liebermann-Burchardt test	Tiwari et al. (2011)
Saponins	EtOH (99%)	Saponin S4521	Frothing test	Scholz & Liebezeit (2006)
Tannins	EtOH (99%)	Tannic acid $C_{76}H_{52}O_{46}$	Gelatine-Saltblock test	Scholz & Liebezeit (2006)
Triterpenoides	EtOH (99%)	18β-Oleanane $C_{30}H_{52}$	Salkowski's Test	LeBlanc et al. (2009)

*Only samples that gave positive reactions to both reagents and tests are assumed to contain alkaloids or aldehydes, respectively

[2]Methyl esters of PUFAs were separated by argentation chromatography using silver nitrate-impregnated thin-layer chromatography plates.

Abbreviations: EtOH: ethanol; MeOH: methanol. PUFA: polyunsaturated fatty acid

4. Results and Discussion

4.1 Cultivable Icelandic Seaweed Species

Overall 16 seaweed species were brought into culture during the present project. In detail, two chlorophyceae (*Ulva lactuca*, *Cladophora arcta*), three phaeophyceae (*Fucus spiralis*, *Fucus vesiculosus*, *Dictyosiphon foeniculaceus*) and four rhodophyceae (*Vertebrata lanosa*, *Gracilaria gracilis*, *Odonthalia dentata*, *Palmaria palmata,* Fig- 13) were grown over their full life cycle beginning with the isolation of spores. In addition, six phaeophyceae (*Saccharina latissima*, *Fucus evanescens, Ascophyllum nodosum, Alaria esculenta, Laminaria digitata, Laminaria hyperborea*) and one rhodophyt (*Delesseria sanguinea*) were stabilized under the conditions described in section 3.3 but with only minor growth (<0.01%) and were therefore not further considered in the present study. With the exception of green alga *Cladophora*, which thallus was not identifiable without doubt, in all further cases it was possible to identify the species using appropriate literature given in section 3.4.

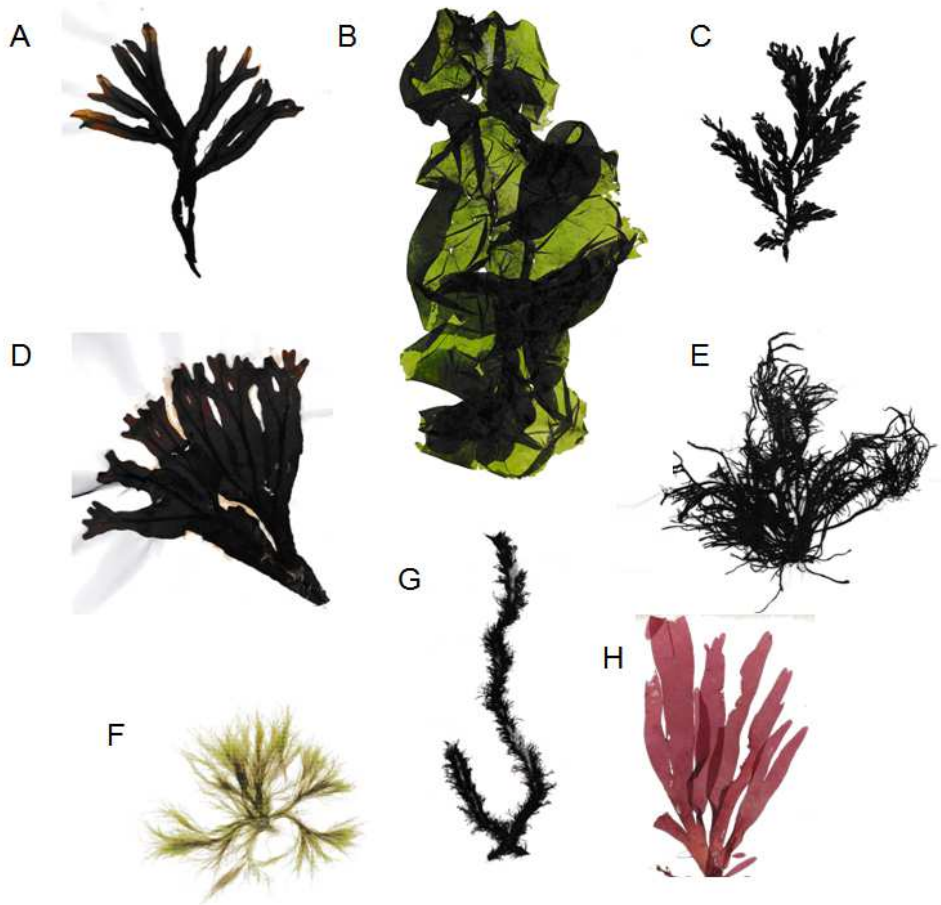

Figure 13. Examples of sporophyte herbaria A) *Fucus spiralis*, B) *Ulva lactuca,* C) *Odonthalia dentata,* D) *Fucus vesiculosus*, E) *Gracilaria gracilis*, F) *Cladophora arcta*, G) *Vertebrata lanosa*, and H) *Palmaria palmata*.

Particularly kelps were found to be dependent on constantly circulating seawater which was not realizable in the BioPol facilities. In addition, far lower temperatures were for several of the other tested seaweed species (including kelps) necessary for stable culturing of taxa. The incubators used during the spore isolation are only to keep low temperatures (the lowest 8°C) over a very limited time period and thus all future attempts for large scale cultivation of cold adapted seaweed species will be only possible in cooperation with institutes with appropriate equipment such as the University College Hólar.

Although spore isolation was successful for the cultivable seaweeds taxa named above, it was not possible to stabilize the spores over time. In all tested cases, spores developed naturally directly into the next growth stage. Thus a build up of a "seed bank" of Icelandic seaweeds will be only possible in combination with the cryopreservation of spores. This procedure is in the moment only available for some seaweed species such as *Laminaria japonica* (Zhang et al. 2007) and is still very experimental. It seems to be possible to use agents such as dimethyl sulphoxide (DMSO), glycerol, sucrose, dextrose and sorbitol which are cryoprotectants effectively employed for the cryopreservation of microalgae in combination with a complex cooling process. But it was found that, after following a process of gradual cooling, 24 h storing in liquid nitrogen and quick thawing, only 50% of cryopreserved spores remained viable (Zhang et al. 2007). Thus further scientific work in this field has to be conduct before a seed bank of Icelandic seaweed species can be realized.

4.2 Growth and Biochemical Gross Compositions

Table 4. Specific growth rate and biochemical gross composition of nine seaweed species as percent dry weight (DW), obtained from cultures grown under standardised growth conditions. Values are means (± SE, n = 12).

Species	**Growth** [% in wet weight per week]	**Protein** [% DW]	**Lipid** [% DW]	**Carbo-hydrate** [% DW]	**Ash** [% DW]
Ulva lactuca	8.5 ± 1.3	16.1 ± 0.9	10.9 ± 0.8	36.4 ± 2.2	24.3 ± 0.2
Cladophora arcta	0.9 ± 0.02	15.7 ± 1.4	8.8 ± 1.1	22.8 ± 0.6	20.5 ± 0.4
Fucus spiralis	0.3 ± 0.06	16.3 ± 1.1	12.4 ± 2.3	28.4 ± 1.1	29.1 ± 0.5
Fucus vesiculosus	0.1 ± 0.05	11.8 ± 0.5	12.9 ± 1.5	35.3 ± 1.0	29.1 ± 0.1
Dictyosiphon foeniculaceus	0.05 ± 0.1	4.1 ± 0.5	8.4 ± 0.7	22.5 ± 0.6	22.4 ± 1.4
Vertebrata lanosa	0.04 ± 0.1	4.4 ± 0.7	12.3 ± 1.6	21.4 ± 2.2	20.1 ± 1.2
Gracilaria gracilis	1.9 ± 0.2	8.9 ± 0.4	18.5 ± 1.2	35.9 ± 1.9	19.9 ± 0.7
Odonthalia dentata	1.1 ± 0.3	11.2 ± 0.4	15.3 ± 1.0	35.1 ± 2.3	21.9 ± 1.6
Palmaria palmata	2.2 ± 0.3	29.7 ± 0.7	22.4 ± 2.1	42.8 ± 1.3	27.6 ± 1.1

Specific growth rates, determined for the different seaweed species during the culture in 2 L Erlenmeyer flasks under optimized standard conditions (cf. Table 2), showed highly species specific characteristics and was the highest for the chlorophyte *Ulva lactuca* followed by the rhodophyte

Palmaria palmata (Table 4). In contrast, seaweed species such as *D. foeniculaceus* and *V. lanosa* showed only minor growth, being around 99% lower as compared to *U. lactuca*. *Vertebrata lanosa* is an abundant and obligate red algal epiphyte of *Ascophyllum nodosum* that forms part of a complex and highly integrated symbiotic system that includes the ascomycete, *Mycophycias ascophylli* (Gabary et al. 2014). In the present study this red alga was grown detached from its symbiont, suggesting that for optimal growth also the integration of further co-cultivated species might be necessary. In all further cases the comparison to existing literature is not possible due to differences in the methods used during cultivation (e.g. line cultures in the environment) or the complete lack of literature regarding some species. For instance, data for different *Fucus* species are available, but in most cases only thallus pieces were cultured over relative short time periods (e.g. Graiff et al. 2015).

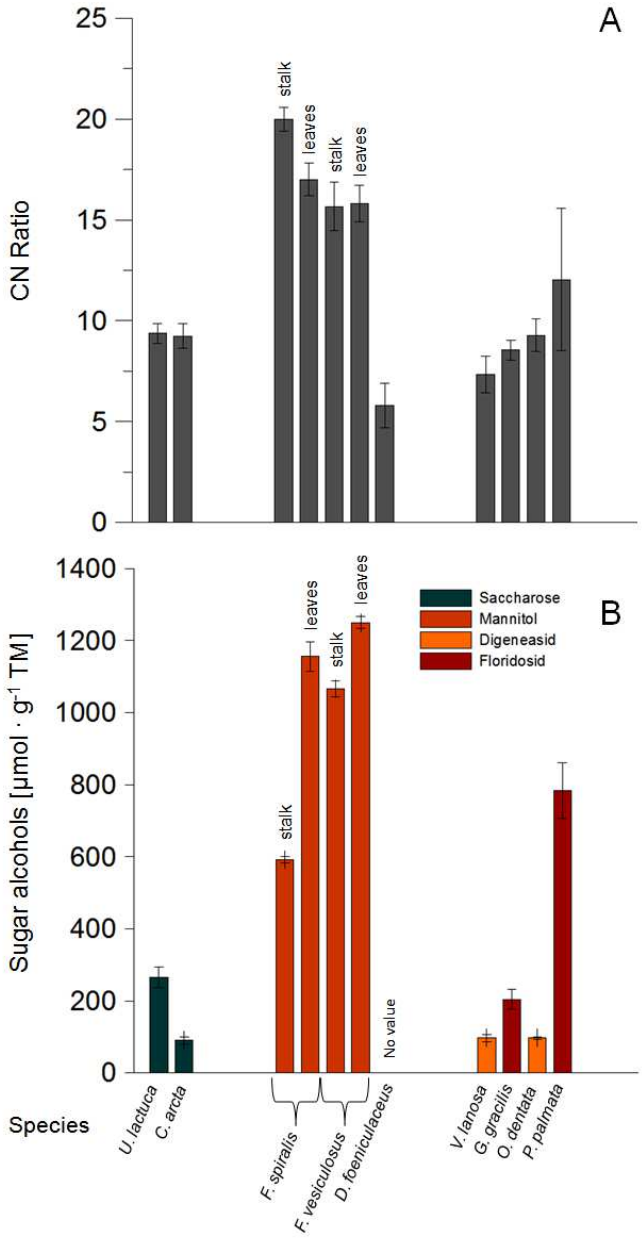

Figure 14. Carbon (C)/nitrogen (N) ratios (A) and sugar alcohols ((B) saccharose, mannitol, digeneasid, floridosid) of the different seaweed species.

The biochemical contents of the nine algal species are also listed in Table 4. Particularly, the biochemical gross compositions of *Ulva lactuca, Palmaria palmata* and *Gracilaria gracilis* are within the range of published literature (e.g. Khairy & El-Shafay 2013; Morgan et al. 1980; Francavilla et al. 2013). In particular, the protein content of seaweeds can vary according to species, seasonal period and geographic area (Fleurence 1999; Haroon 2000; Ratanaarporn & Chirapart 2006). Wong & Cheung (2000) recorded similar values in the red alga *Palmaria* sp. (13.87%), as well as conspicuously lower values in the green alga *U. lactuca* (7.06%). Similarly changes in carbohydrate and lipid contents were observed to be season depending and thus are susceptible to manipulation by variation of culture conditions such as temperature, nutrients or light intensities (Khairy & El-Shafay 2013).

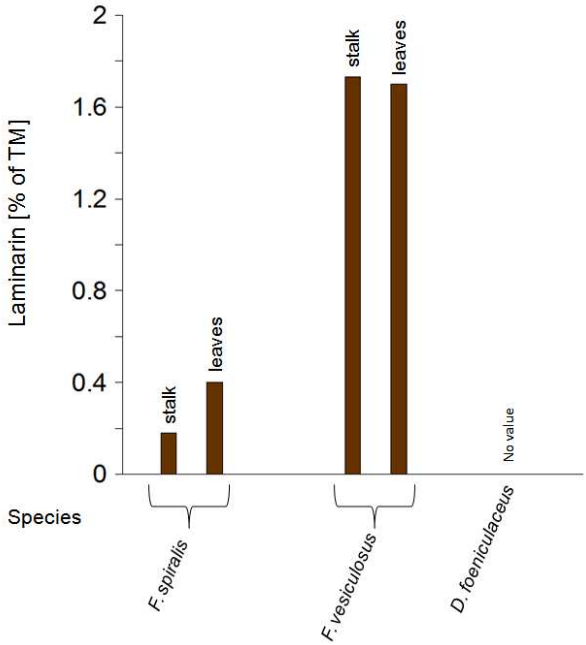

Figure 15. Laminarin contents of *Fucus spiralis* and *F. vesiculosus* (stalk and leaves).

4.3 Utilizable Compounds in Icelandic Seaweeds

Carbon/Nitrogen (C:N) ratios are useful in interpreting the physiological state of algae and are usually closely correlated to the main products of photosynthesis such as mannitol or (Fig. 14). It was found that CN ratios and the accumulation of storage products are directly related to environmental conditions such as temperature. For instance, Graiff et al. (2015) found that the concentration of the main carbohydrate of photosynthesis in brown algae (mannitol) and also the molar CN ratio increased with higher temperatures and the response curve of mannitol and CN content followed that of photosynthesis. A similar correlation was in the present study only observed for *Palmaria palmata* and its storage compound floridosid and in weaker form in both chlorophytes (*U. lactuca* and *C. arcta*, storage product saccharose) and the rhodophytes *Vertebrata lanosa*, *Gracilaria gracilis* and *Odonthalia dentata*. In contrast, in both *Fucus* species the highest mannitol contents were recorded in the leaves, while in the stalks the contents were significantly lower (51% in *F. spiralis*). In

addition, the CN ratios of both *Fucus* species were not positively correlated with their storage compound mannitol and laminarin.

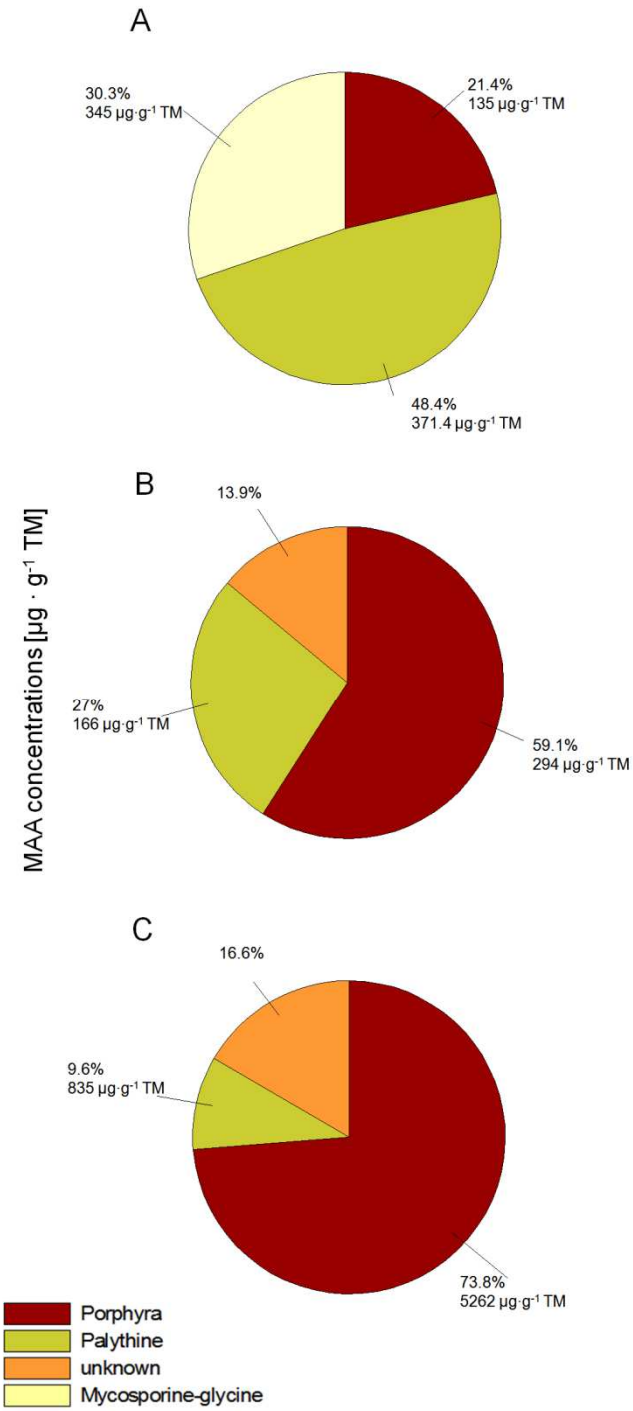

Figure 16. MAA concentrations of *Vertebrata lanosa* (A), *Gracilaria gracilis* (B), and *Palmaria palmata* (C), including percentage values and concentrations of the MAAs porphyra, palythine and mycosporine-glycine, including values for an unknown MAA.

Furthermore, in *Dictyosiphon foeniculaceus* neither mannitol nor laminarin (Fig. 15) was found. One explanation might be that the concentration of these storage products were under the detection limit but due to the relative high carbohydrate content (Table 4) it is highly likely that the main

product of photosynthesis might be another storage compound. Particularly, the polysaccharide laminarin (β-1,3-glucan) is used as a long-term carbon storage compound in brown algae. This chemical storage form of carbon enables perennial brown algae in seasonally fluctuating ecosystems to uncouple growth from photosynthesis, i.e., most of these plants grow as seasonal anticipators in winter based on remobilization of laminarin, while in summer, growth typically ceased to fill up the storage pool. Brown algae lack or contain only very low concentrations of free sugars such as glucose, fructose, or sucrose (Yamaguchi et al. 1966). These free sugars are supposed to undergo immediate biochemical conversion into D-mannitol and polysaccharides. The long-term carbon storage polysaccharide in brown algae is laminarin (β-1,3-glucan), which chemically markedly differs from storage products of most other living organisms that typically use glycogen or starch (α-1,4-glucans). Laminarin was first described by Schmiedeberg (1885), who isolated it from Laminariaceae. It consists of chains of β-1,3'-linked glucose units with occasional β-1,6'-linkages (Percival & Ross 1951; Beattie et al. 1961). These β-1,6'-linkages are present in a linear chain of β-1,3'-linked glucose residues and mainly as interchain linkages which lead to a ramification of the molecule (Peat et al. 1958; Annan et al. 1965). The branching factor determines water solubility; the higher the branching content, the higher the solubility in cold water (Annan et al. 1965). The laminarin contents in the present study showed the highest values in the leaves and stalks of *Fucus vesiculosus*, whereas in *Fucus spiralis* the contents were up to 76% lower.

Vertebrata lanosa, *Gracilaria gracilis* and *Palmaria palmata* extracts exhibited in each case three main MAAs (Fig. 16). While in *G. gracilis* and *P. palmata* the MAA porphyra-334 was found to exhibit the highest concentration (up to 5262 $\mu g \cdot g^{-1}$ TM in *P. palmata*), the main MAA in *V. lanosa* was palythine. In addition, only in *V. lanosa* was the MAA mycosporine-glycine detectable. The presence of one unidentifiable compound in *G. gracilis* and *P. palmata* is noteworthy and needs further scientific studies using for example nuclear magnetic resonance spectroscopy (NMR).

4.4 Further interesting Compounds Classes

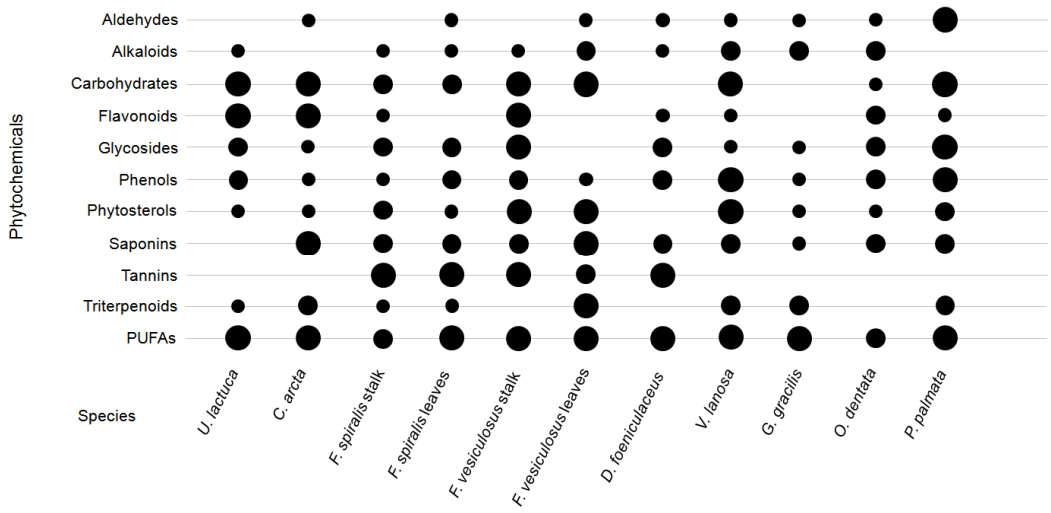

Figure 17. Results of the phytochemical screening, targeting several different compound classes with pharmacological relevance such as tannins, saponins, flavonoids or phytosterols.

Several for pharmaceutical applications interesting compound classes were found during the phytochemical screening of the nine Icelandic seaweed species (Fig. 17). Important phytochemicals, such as alkaloids, triterpenoids, steroids, tannins, saponins, phytosterols, and flavonoids were found to be present in the extracts, but differed in their concentrations between the tested species. Based on these concentration differences the most promising seaweed species were *Fucus vesiculosus* and *Palmaria palmata*.

Particularly, phenolic compounds are commonly found in plants, including seaweeds, and have been reported to show a wide range of biological activities including antioxidant properties (Athukorala et al. 2006; Kuda et al. 2007). Reports have revealed that phenolic compounds are one of the most effective antioxidants in red algae. Viswanathan et al. (2014), reported crude methanolic extracts of red seaweeds to yield results in the range of 1.5–4.1 mg GAE/g, which is higher in phenolic content than the red species studied in this work. The present study was promising, as algae polyphenolic compounds are effective antioxidants in delaying oil rancidity, and therefore, the seaweed extracts could have a potential effect in food application. Total flavonoids in the seaweeds ranged from 0.104 to 0.332 mg/g. Kahkonen et al. (1999) stated that flavonoids are probably the most important natural phenol due to their broad spectrum of chemical and biological activities, including antioxidant and free radical scavenging properties. Flavonoids have been reported as antioxidants of a wide range of reactive oxygen species and inhibitors of lipid peroxidation and as potential therapeutic agents against a wide variety of diseases. It has been reported that the presence of phytoconstituents such as flavonoids, tannins and polyphenols prevent a number of diseases through their free radical scavenging activity (Duan et al. 2006), and these phenolic compounds, which include phenol, tannin and flavonoids, have been found in appreciable amounts in the tested seaweed species.

Further studies are required to investigate the extracts for their potential pharmacological properties. The present study suggests that the seaweed extracts possessed phytochemical activity thus supporting their folkloric usage, promising a future scope for the use of these marine seaweeds against microbial contaminants.

5. Acknowledgements

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7. Appendix

Chemicals

If not otherwise mentioned, all of the chemicals used in this study were of the highest purity from Sigma/Aldrich.

Media receipts

VON STOSCH'S ENRICHED SEAWATER MEDIUM

von Stosch's Enrichment (as cited by Ott, 1966)

The seawater should be filtered (Whatman's #1) to remove large organic particles and sand. Then sterilize by autoclaving (time: 100 ml requires 10 minutes; 2 liters requires 40 minutes; 3 liters requires 50 minutes; and 5 liters requires 70 minutes).
To each liter of seawater, then add the following:

Salts	1 liter of seawater
(1) $NaNO_3$	42.50 mg
(2) $Na_2HPO_4 \cdot 12H_2O$	10.75 mg
(3) $FeSO_4 \cdot 7H_2O$	278.00 µg
(4) $MnCl_2 \cdot 4H_2O$	19.80 µg
(5) $Na_2EDTA \cdot 2H_2O$	3.72 mg
Vitamins	
(6a) Thiamine-HCl	0.20 mg
(6b) Biotin	1.00 µg
(6c) B_{12}	1.00 µg

It is convenient to prepare a stock solution of each salt in distilled water; of such concentration that 1 ml of the stock solution gives the required concentration of each ingredient. The three vitamins may be incorporated in the same stock solution, which should be refrigerated. The salts and vitamins after preparation into stock solutions should be filter sterilized.

I. To make stock solutions use deionized distilled water and clean volumetric flasks.

II. Filter each stock solution through separate 0.22 µm Millipore filters. Each solution will have to be sterilized separately.

III. Aseptically pour filtered volume of liquid into autoclaved stock bottles.

1-liter stock solution	2-liter stock solution
(1) 42.500 grams	85.000 grams
(2) 10.750	21.500
(3) 0.278	0.556
(4) 0.0198	0.039
(5) 3.720	7.440
(6a) 0.200	0.400
(6b) 0.001	0.002
(6c) 0.001	0.002

f/2 Medium

(Guillard and Ryther 1962, Guillard 1975)

Component	Stock Solution	Quantity	Molar Concentration in Final Medium
$NaNO_3$	75 g/L dH_2O	1 mL	8.82×10^{-4} M
NaH_2PO_4 H_2O	5 g/L dH_2O	1 mL	3.62×10^{-5} M

Na_2SiO_3 $9H_2O$	30 g/L dH_2O	1 mL	1.06 x 10^{-4} M
trace metal solution	(see recipe below)	1 mL	---
vitamin solution	(see recipe below)	0.5 mL	---

f/2 Trace Metal Solution

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
$FeCl_3$ $6H_2O$	---	3.15 g	1.17 x 10^{-5} M
Na_2EDTA $2H_2O$	---	4.36 g	1.17 x 10^{-5} M
$CuSO_4$ $5H_2O$	9.8 g/L dH_2O	1 mL	3.93 x 10^{-8} M
Na_2MoO_4 $2H_2O$	6.3 g/L dH_2O	1 mL	2.60 x 10^{-8} M
ZnSO4 $7H_2O$	22.0 g/L dH_2O	1 mL	7.65 x 10^{-8} M
$CoCl_2$ $6H_2O$	10.0 g/L dH_2O	1 mL	4.20 x 10^{-8} M
$MnCl_2$ $4H_2O$	180.0 g/L dH_2O	1 mL	9.10 x 10^{-7} M

f/2 Vitamin Solution

Component	Primary Stock Solution	Quantity	Molar Concentration in Final Medium
thiamine HCl (vit. B_1)	---	200 mg	2.96 x 10^{-7} M
biotin (vit. H)	0.1 g/L dH_2O	10 mL	2.05 x 10^{-9} M
cyanocobalamin (vit. B_{12})	1.0 g/L dH_2O	1 mL	3.69 x 10^{-10} M

Modified Provasoli

Stocks **per litre**

(1) PII trace metals

Na_2 EDTA	1.0 g
H_3BO_3	1.12 g
$MnSO_4.H_2O$	0.12 g
$ZnSO_4.7H_2O$	0.022 g
$CoSO_4.7H2O$	0.005 g

(2) Iron-EDTA

$Fe(NH_4)_2(SO_4)_2.6H_2O$	0.7 g
Na_2 EDTA	0.6 g

Medium	**Stock**	**per litre medium**
Na_2 ß-glycero $PO_4.5H_2O$	50 g/litre	8.0 ml
$NaNO_3$	35 g/litre	110 ml
Iron-EDTA (2)		100 ml
Vitamin B_{12}	0.01 g/litre	8.75 ml
Thiamine	0.5 g/litre	8.0 ml
Biotin	0.005 g/litre	8.0 ml
PII trace metals (1)		200 ml

To prepare final medium, dispense the above into 10 ml aliquots and sterilize by autoclaving. Finally, to use add 10 ml per litre to sterile 30 ppt filtered seawater.

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