Optimisation of the sequential extraction of fucoidan and sodium alginate from a Southern African brown seaweed, *Ecklonia maxima*

A thesis submitted in fulfilment of the requirements for the degree of

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by

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Abstract

Seaweeds have been used in many industries for their biochemical properties, including as bioactive compounds for pharmaceuticals and nutraceuticals. Seaweeds have been shown to contain various valuable bioactive compounds, such as hydrocolloids, polyphenols and carotenoids. These chemical compounds exhibit different bioactivities, including anti-inflammatory, anti-microbial, anti-oxidant, anti-cancer, anti-diabetic and anti-HIV activity. Furthermore, a recent review in 2020 revealed an increase in the number of studies conducted on alginate and other polysaccharides over the past decade due to a growing awareness of their use as sustainable biomaterials. Therefore, there is a growing demand for polysaccharides from algae, such as alginate, and sulphated polysaccharides, such as fucoidan. The brown seaweed *Ecklonia maxima*, which is endemic to South Africa, is abundant but underexploited for its bioactive compounds. This study was conducted to optimise various parameters to co-extract fucoidan and sodium alginate and to compare the chemical and physical characteristics of the extracts with those of commercially available fucoidan and alginate.

During the first stage of this study, the acidic co-extraction of fucoidan and sodium alginate from *Ecklonia maxima* was optimised. The optimised parameters for the co-extraction procedure were as follow: pH 1.0 for the delipidation step, 80% (v/v) ethanol for fucoidan precipitation, 0.5 M sodium carbonate addition for converting alginate into sodium alginate, and 70% (v/v) ethanol for sodium alginate precipitation. This method successfully co-extracted fucoidan and sodium alginate with yields of 3.67 and 58.7% (w/w dry mass basis), respectively - from a starting material of 7.5 g. The yield of sodium alginate was greater than any yield reported in the literature. The optimised method was scaled up using an increased starting mass of seaweed, up to 200 g, for the large-scale (LS) extraction.

The LS extracts were examined for their purity, chemical composition and physical characteristics, such as viscosity, molecular weight, and structural conformation, compared to small-scale (SS) extracts and commercially available fucoidan and alginate standards. The chemical and physical characteristics of the LS and SS extracts were very similar. The extracts contained almost no contaminants, such as phenolics and proteins, when they were evaluated for purity. The chemical compositions of the extracts were similar to those of their commercial counterparts. Both the commercial standards and LS fucoidan were shown to contain large amounts of D-glucose, L-fucose, and sulphate. In contrast, sodium alginates contained large amounts of D-glucuronic acid, D-mannose, and total uronic acid. Carbazole was used to

determine total uronic acid in sodium alginates, which were present in high concentrations for all the sodium alginates; this confirmed that the sodium alginate extracts consisted primarily of uronic acid.

Various methods were used to determine the physical characteristics of the fucoidan and sodium alginate, such as Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), circular dichroism (CD) spectroscopy, molecular weight determination, Congo red, and X-ray diffraction (XRD). FTIR indicated that all the required peaks were present, and the extracted fucoidans and sodium alginates displayed the same FTIR profile typical for the commercial fucoidan and sodium alginates standards, confirming the purity and chemical composition of the extracted compounds. CD spectroscopy indicated a mannuronic to guluronic (M/G) ratio of 1.91 for the LS sodium alginate and 0.76 for the commercial sodium alginate. The viscosity average molecular weight of LS sodium alginate was 447 kDa using the Mark-Houwink-Sakurada equation. Both *E. maxima* sodium alginates and commercial sodium alginate were suspected of assuming a triple-helical conformation in solution and were amorphous in structure, as confirmed by Congo red and XRD analysis, respectively.

In conclusion, this study optimised the co-extraction of fucoidan and sodium alginate from brown seaweed and scaled up the extraction process more than 25-fold with a high level of reproducibility. The SS and LS fucoidan and sodium extracts were confirmed to have very similar chemical and physical characteristics to each other, as well as to the commercial fucoidan and sodium alginate standards. The extracted sodium alginate also had a high viscosity, a high molecular weight, and a high M/G ratio. The sequential and combined extraction method for fucoidan and sodium alginate proposed in this study is indeed a feasible and robust approach and can make a significant contribution to the seaweed biorefinery and greater bioeconomy.

Declaration

I, Yuchan Park, declare that this thesis is my unaided work. It is hereby submitted for the degree of Master of Science from the Faculty of Science, Rhodes University. It has not been submitted before for any degree or examination at any other university.

Date: January 2025

Signature: Yuchan Park

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List of abbreviations

°C	Degree Celsius
µg	Microgram
$\mu g.ml^{-1}$	Microgram per milliliter
µM	Micromolar
µmol	Micromole
ANOVA	Analysis of Variance
BSA	Bovine serum Albumin
DNS	Dinitrosalicylic
D_2O	Deuterium Oxide
EAE	Enzyme-assisted extraction
FT-IR	Fourier-transform infrared spectroscopy
g	Gram
HCl	Hydrochloric acid
HIV	Human immunodeficiency virus
kDa	Kilo Dalton
KOH	Potassium hydroxide
LS	Large-scale
mg	Milligram
$mg.ml^{-1}$	Milligram per millilitre
ml	Millilitre
min	Minute
MAE	Microwave-assisted extraction
MHz	Megahertz
Mw	Molecular weight
nm	Nanometer
NMR	Nuclear magnetic resonance
SS	Small-scale
TMS	Tetramethylsilane
UAE	Ultrasound-assisted extraction
v/v	Volume per volume
w/v	Weight per volume
w/w	Weight per weight
XRD	X-ray powder diffraction

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List of outputs emanating from this study

Publication in international peer-reviewed journals:

Park, Y., Malgas, S., Krause, R.W.M., Pletschke, B.I.* (2024) Extraction and characterisation of sodium alginate from the Southern African seaweed *Ecklonia maxima. Botanica Marina* 67 (5), 513-523.

International Conference Proceeding:

Mabate, B., Daub, C.D., Park, Y. and Pletschke, B.I.* (2023) Extracting bioproducts from kelp biomass for the blue biotechnology. (*Plenary Lead Speaker). International Conference on Exploring New Horizons in Biotechnology (ENB-2023). Banaras Hindu University, Varanasi, UP, India. 10th-12th February, 2023

Chapter 1 – General Introduction and Literature Review

1.1 Introduction

For centuries, seaweeds have been used and consumed by many Asian countries for their beneficial health effects; however, there is no evidence that African countries have been growing or cultivating seaweed for human consumption (Stirk *et al.,* 2004; Marsham *et al.,* 2007; Rioux *et al.,* 2009). Seaweeds range from unicellular to multicellular forms and are mainly autotrophic organisms. They are categorised into three phyla, namely Rhodophyta (red algae), Phaeophyceae (brown algae) and Chlorophyta (green algae), which are differentiated by their chemical properties (Dawczynski *et al.,* 2007). This makes seaweed diverse organisms that can grow in large amounts and be found across oceans worldwide. In recent years, seaweeds have been utilised in many industries for their chemical properties, including the extraction of bioactive compounds for pharmaceuticals and nutraceuticals and as thickening and stabilising agents for cosmetics and in food industries (Cardozo *et al.,* 2007; Holdt and Kraan, 2011). The chemical compounds in seaweeds have been shown to contain various bioactive compounds, such as hydrocolloids, polyphenols and carotenoids. These chemical compounds show multiple bioactivities, including anti-inflammatory, antimicrobial, antioxidant, anti-cancer, anti-diabetes and anti-HIV activity (Manilal *et al.,* 2009). The broad spectrum of bioactivities in seaweeds makes them valuable for pharmaceutical and nutraceutical products.

Seaweed farming was introduced to South Africa in the late 20th century. A few seaweed species have been farmed and cultivated for commercial use since, with most of the farming activity occurring on the west coast of southern Africa (Stirk *et al.,* 2004). South Africa accounts for only 2% of the world's wild seaweed harvest, but it is one of the leading producers outside Asia (Nayar and Bott, 2014). However, South Africa must still utilise the full potential of seaweeds harvested in its oceans. The seaweeds in South Africa are cultivated mainly for use as abalone feed and agricultural plant growth stimulants (Stirk *et al.,* 2004). According to Rothman *et al.* (2020), 3005 tonnes of fresh *E. maxima* were harvested in 2017 to be used as agricultural plant growth stimulants, while 7000 tonnes of fresh kelp were harvested in 2011 for abalone feed. For bioactive compounds in South Africa, about 1000 tonnes of dry-weight seaweed per year has been used to produce

alginate for commercial purposes (Anderson *et al.,* 2007). The main reason for the low production of alginate or other bioactive compounds from seaweeds is the lack of expertise and limited techniques to extract the bioactive compounds efficiently. However, with recent advances in the extraction of bioactive compounds from seaweeds, the bio-industrial value of seaweeds from South Africa and their economic value hold excellent potential (Daub *et al.,* 2020; Nasab *et al.,* 2020).

1.2 Compounds in seaweeds

1.2.1 Hydrocolloids

1.2.1.1 Agar

Agar is composed of a mixture of polysaccharides, including agarose and agropectin, and has characteristics similar to carrageenans. Agarose comprises β-1,3-linked D-galactosyl and α-1,4-linked 3, 6-anhydro-L-galactosyl residues (Zhang *et al.,* 2004) (Figure 1.1). On the other hand, agaropectin is made up of D-galactosyl and L-galactosyl residues (Rhein-Knudsen and Meyer, 2021). The composition of agar and agaropectin depends on the seaweed type and the extraction method used (Rhein-Knudsen and Meyer, 2021). Souza *et al.* (2012) have reported an agar yield with a composition of 65% galactose, 25% 3, 6-anhydrogalactose and 8% sulphate from *Gracilaria birdiae* using reductive acid hydrolysis (90 °C, 45 min). In another study by Rodríguez *et al.* (2009), they extracted agar with a composition of 28% galactose, 35% 3, 6-anhydrogalactose and 4% sulphate from *Gracilaria gracilis*. Agar can be easily extracted from red seaweeds, such as *Gelidium spp.* and *Gracilaria spp.*, which have been shown to contain up to 32 - 35% agar on a dry mass basis (Rasmussen and Morrissey, 2007).

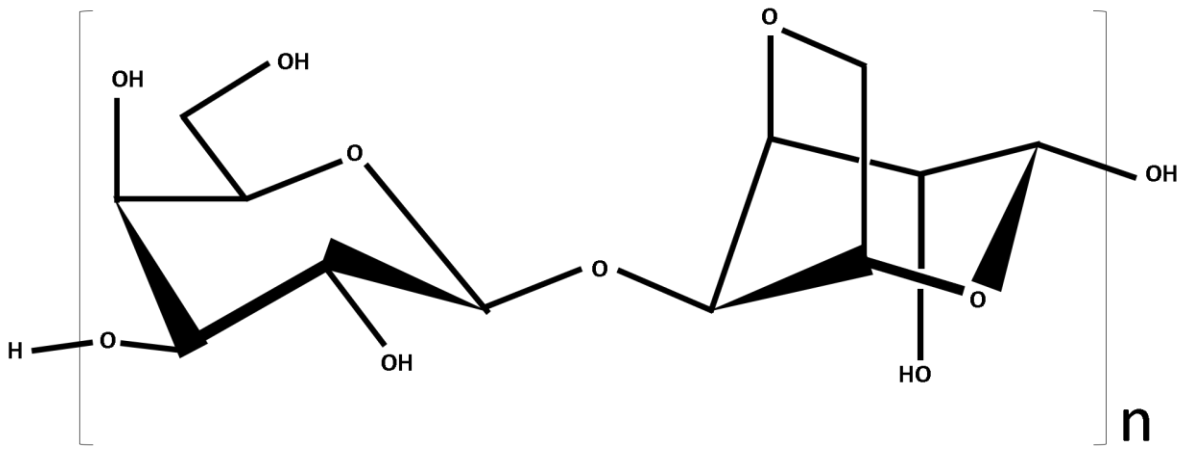
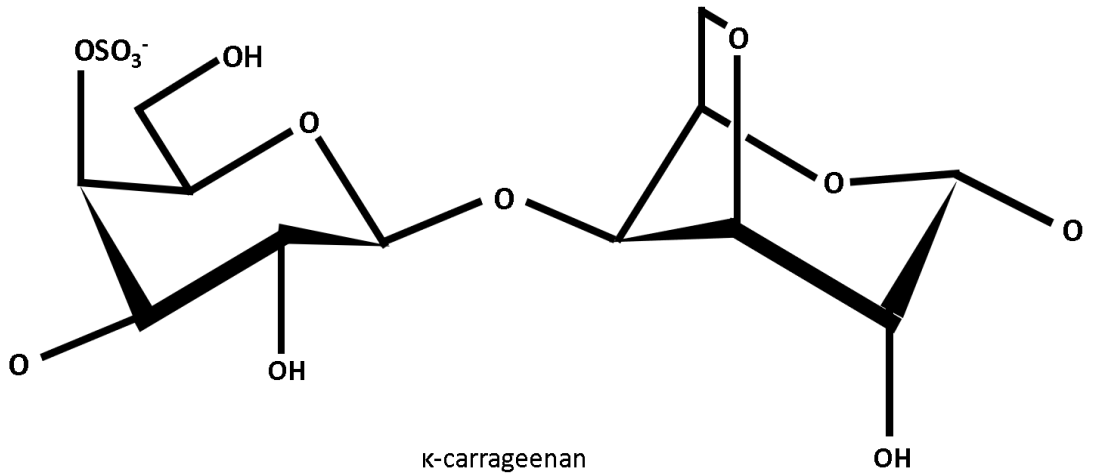


Figure 1.1: An illustrated structure of agarose repeating units.

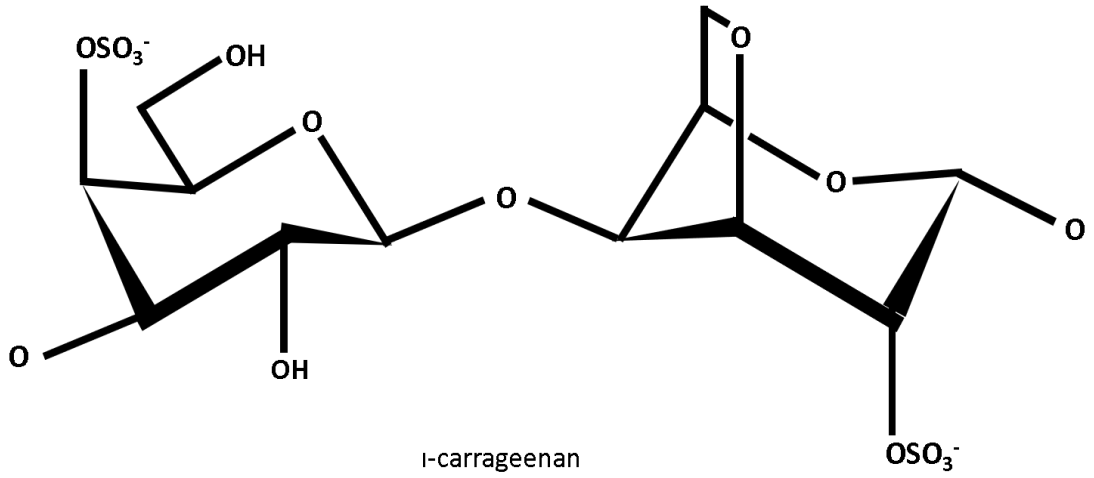
According to Pal *et al.* (2014) and Romero *et al.* (2008), agar is divided into low, medium and high-quality agar, with gel strength increasing with quality. The low-quality agar is used in food products, such as sweets, frozen foods, fruit juice, bakery icing and industrial applications; paper coating, adhesives, textile printing, dyeing and casting (Armisen and Galatas, 1987; Pal *et al.,* 2014). On the other hand, medium-quality agar is used in biological culture media, bulking, anticoagulant agents, laxatives, capsules, and tablets in pharmaceutical industries (Pal *et al.,* 2014). In the form of agarose, high-quality or highly purified agar is currently used in molecular biology for electrophoresis, immunodiffusion and gel chromatography (Pal *et al.,* 2014). Agar has also been reported for its bioactive properties; for example, Holdt and Kraan (2010) reported that agar decreases blood glucose levels and exhibits an anti-aggregation effect on red blood cells. Agar-derived oligosaccharides have been used to treat cancer cells since they induce apoptosis of cancer cells *in vitro* and have also been shown to suppress the production of a pro-inflammatory cytokine and caspase, an enzyme linked to the production of nitric oxide (Enoki *et al.,* 2003; Chen *et al.,* 2005; Holdt and Kraan, 2011). According to Rhein-Knudsen *et al.* (2015), agar is the most expensive hydrocolloid, estimated at USD 18 per kg, while carrageenan and alginate cost USD 18 and USD 12 per kg, respectively.

1.2.1.2 Carrageenan

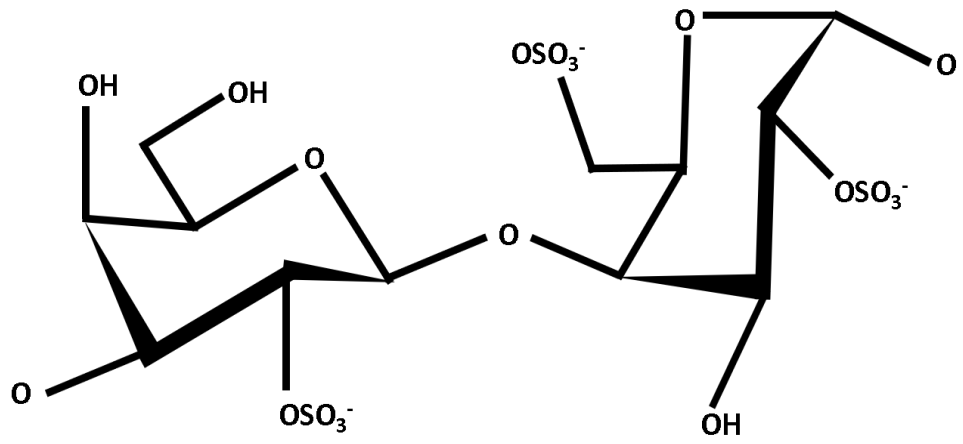
Carrageenan is a hydrocolloid made up of linear polysaccharide chains of alternating β-D-galactose and α-D-3,6-anhydrogalactose residues with sulphate esters attached to them (Rochas *et al.,* 1989). According to their gelling properties, carrageenans are divided into three general groups: kappa, iota and lambda carrageenans (Figure 1.2) (Rasmussen and Morrissey, 2007). Kappa carrageenan has been reported to form strong gels, while iota carrageenan forms soft gels and lambda carrageenan gels rapidly (McHugh, 2003). Carrageenan solubilises in water over a wide pH range to develop a viscous solution (Holdt and Kraan, 2011). This characteristic makes carrageenan a valuable resource in many industries. Carrageenan is used in canned foods, salad dressings and milk products, such as chocolate pudding, dessert gels, ice creams, jellies and jams (Holdt and Kraan, 2011; Pal *et al.,* 2014). As an alternative fining agent, carrageenan is also used during brewing to clarify beer and wine (Holdt and Kraan, 2011). The pharmaceutical industry has also reported its use in drug delivery systems, where it's used as a matrix to control human drug release (Rhein-Knudsen *et al.,* 2015). In medicine, carrageenan has been shown to prevent the attachment of viruses to cells, such as the human papillomavirus, dengue virus and herpes virus (Buck *et al*., 2006; Li *et al.,* 2014). Carrageenan has also been reported to have potential anti-tumour, anti-viral and anticoagulant properties (Zhou *et al.,* 2004; Zhou *et al.,* 2006). Carrageenan can be found in red algae, such as *Chondrus Crispus* and *Kappaphycus spp*., which contain up to 71% and 88% of carrageenan on a dry mass basis, respectively (Rodriguez *et al.,* 2007).



κ-carrageenan



ι-carrageenan



λ-carrageenan

Figure 1.2: Illustrated structures of κ-, ί- and λ-carrageenans.

1.2.1.3 Alginate

Alginate is a linear polysaccharide containing 1, 4-linked β-D-mannuronic and α-L-guluronic acid residues (Figure 1.3) (Pal *et al.,* 2014). Alginate is mainly distinguished from other hydrocolloids as it is extracted from brown seaweeds instead of red seaweeds. In brown seaweeds, it is present in the intercellular space matrix of the seaweed, where it's involved in transporting calcium, magnesium and sodium salts in and out of the cells (McHugh, 2003; Rhein-Knudsen *et al.,* 2015). Alginate can comprise up to 40 - 47% of the dry matter of seaweeds, making it one of the most abundant compounds (Rasmussen and Morrissey, 2007; Pal *et al.,* 2014). Rhein-Knudsen *et al.* (2015) have reported that the global market value for alginate is estimated to be USD 339 million. Its market share has increased by 20% in the food and pharmaceutical sectors.

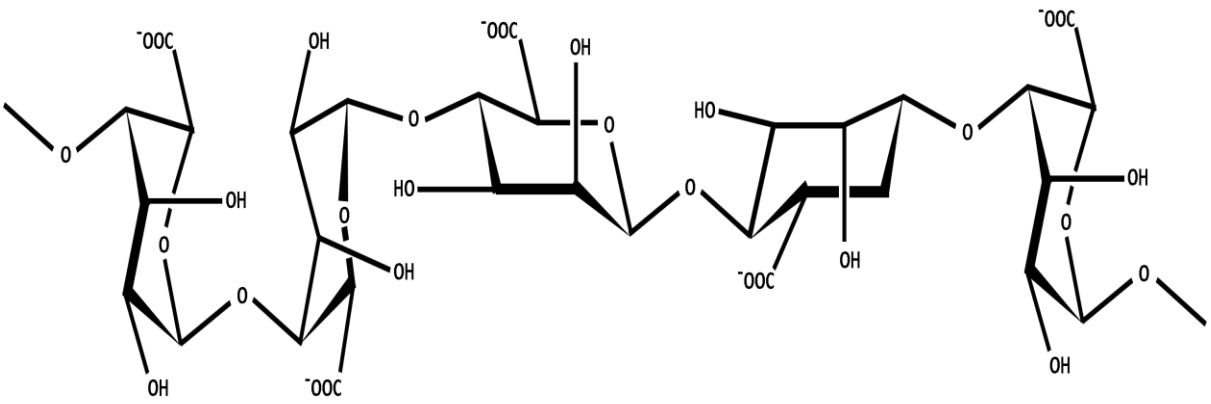


Figure 1.3: An illustrated structure of sodium alginate.

As with other hydrocolloids, alginate also has numerous applications in various industries. Alginate is used in the food industry for its gelling property and ability to chelate metal ions and form viscous liquids in jelly, drinks and desserts (Pal *et al.,* 2014). In the pharmaceutical industry, alginate is used for wound dressing and as a capsules for pills (Paul and Sharma, 2004; Finotelli *et al.,* 2010; Leslie *et al.,* 2013; Pal *et al.,* 2014). Alginate has been reported to decrease animal cholesterol concentrations and is an antibiotic (Nishide and Uchida, 2003). It has also been suggested that the cell wall constituents of brown seaweeds, such as alginate and fucoidan, could absorb heavy metals and chelate heavy metals, such as cadmium and lead, from wastewater (Davis *et al.,* 2003).

Furthermore, alginate salts (sodium alginate and calcium alginate) and alginic acid (the acid form of alginate) have been shown to decrease cholesterol concentration, have a strong antibacterial character, exhibit anti-inflammatory activity without any toxic effects in humans, and have been found to prevent the postprandial increase of glucose, insulin and C-peptide levels (Torsdottir *et al.,* 1991; Holdt and Kraan, 2011). Sodium alginate has also been used in a powder form on its own or mixed with other drugs on septic wounds. It also displays protective and coating effects, shielding mucous membranes and damaged skin against irritation from unfavourable environments (Holdt and Kraan, 2011).

1.2.2 Polysaccharides

1.2.2.1 Fucoidan

Fucoidans are a group of polysaccharides mainly found in the cell walls of brown seaweeds, which can make up more than 40% of algal cell walls on a dry mass basis (Berteau and Mulloy, 2003; Holdt and Kraan, 2011). The fucoidan structure comprises fucose residues substituted with sulphated esters (Li *et al.,* 2008) (Figure 1.4). Moreover, the structure can be complex, as it contains other monosaccharides, such as mannose, galactose, glucose, xylose and uronic acids, acetyl groups and proteins (Wang *et al.,* 2020). Fucoidans are known to prevent seaweed cell walls from drying out during low tide (Holdt and Kraan, 2011).

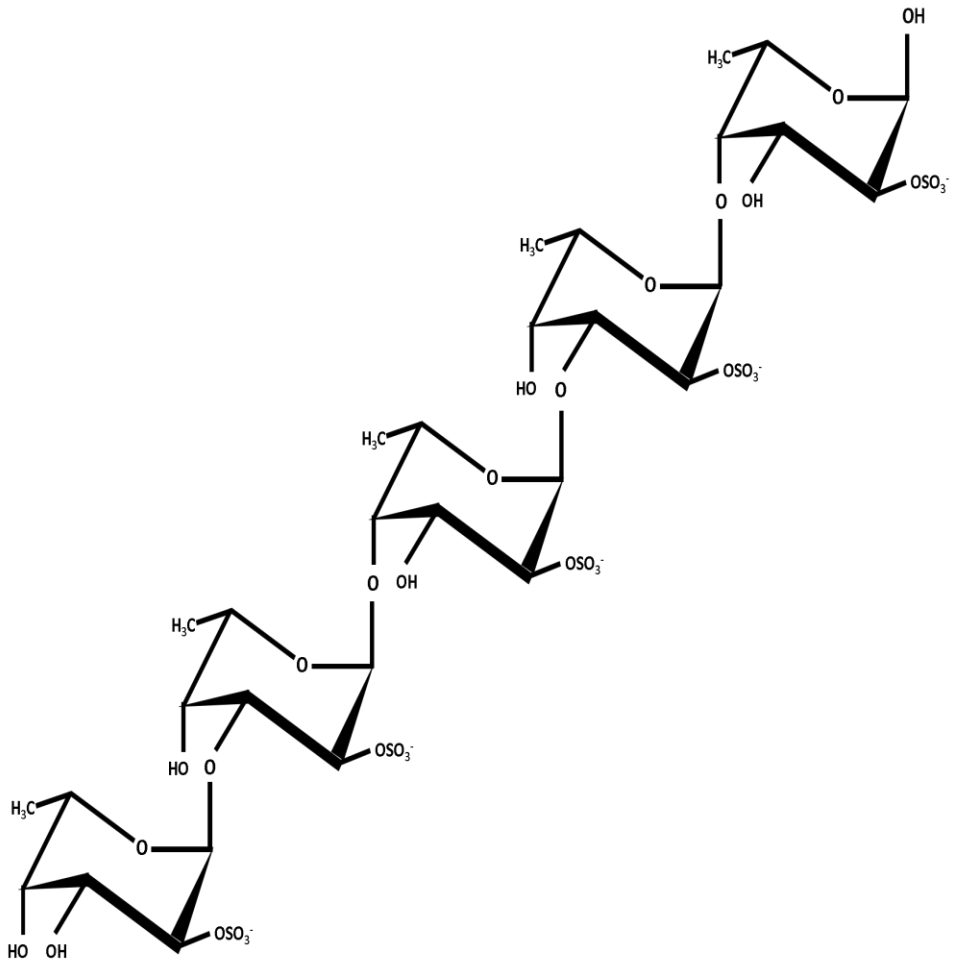


Figure 1.4: An illustrated structure of fucoidan.

According to Berteau and Mulloy (2003), the principal physiological purpose of fucoidans is not certain; however, many reports have shown the beneficial effects of fucoidans on human health. Berteau and Mulloy (2003) have reported that fucoidans can act as injectable anticoagulants. Fucoidans have also been shown to be effective as anti-viral, anti-diabetic, antimicrobial, anti-tumour, anti-inflammatory agents and have even found use as a contraceptive (Cardoso *et al.,* 2014; Daub *et al.,* 2020; Mabate *et al.,* 2021).

1.2.3 Carotenoids

1.2.3.1 Fucoxanthin

Fucoxanthin is a type of carotenoid that is found in brown algae. Fucoxanthin contains an allenic bond, 5, 6-monoepoxide, and nine conjugated double bonds, which are not found in other carotenoids (Figure 1.5) (Zhang *et al.,* 2015). Fucoxanthin is an accessory pigment typically found in the chloroplasts of brown algae, giving them a brown colour (Wijesinghe and Jeon, 2012). Fucoxanthin has been used in the food industry as a supplement and in the cosmetic industry for its pigment (Pereira, 2018). In recent years, fucoxanthin has been shown to have many health effects on the human body, such as inhibition of tumour activity, and its antibacterial, anti-obesity, antioxidant, anti-diabetes and anti-Alzheimer's disease properties (D'Orazio *et al.,* 2012; Hussain *et al.,* 2016; Lin *et al.,* 2016). These properties make fucoxanthin an attractive bioactive compound for the nutraceutical and pharmaceutical industries.

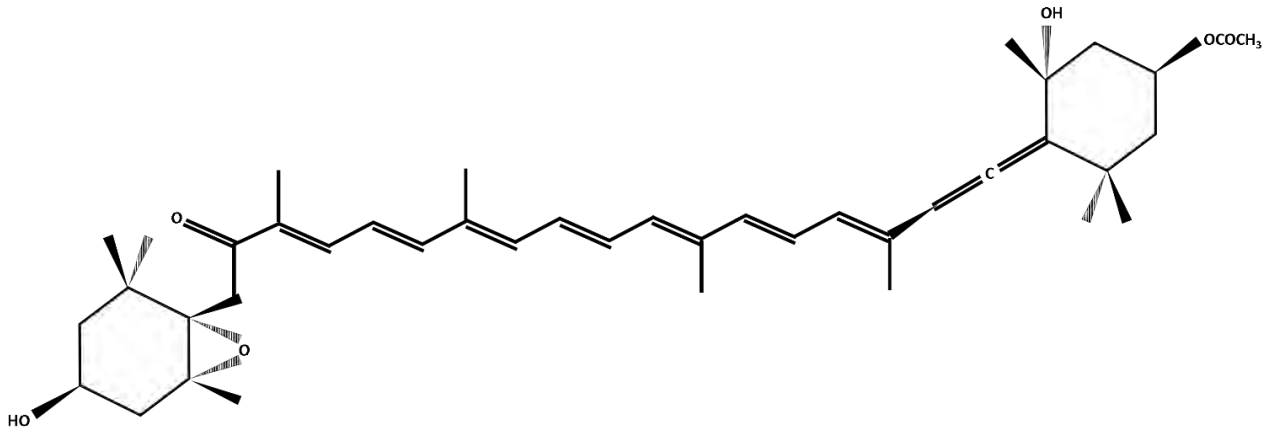


Figure 1.5: An illustrated structure of fucoxanthin.

1.2.4 Polyphenols

1.2.4.1 Phlorotannins

Phlorotannins are highly hydrophilic polyphenols found in many brown algae, such as *Laminaria japonica*, *Ecklonia spp*., *Undaria pinnatifida* and *Hizikia fusiformis* (Kim, 2012). Phlorotannins are biosynthesised via the polyketide pathway by polymerising phloroglucinol monomer units (Poojary *et al.,* 2016). Phlorotannins are separated into phlorethols, fuhalols, fucols,

fucophlorethols, eckols and carmalols (Figure 1.6), depending on the degree of polymerisation of phloroglucinol units and the location of hydroxyl groups (Lopes *et al.,* 2016). Phlorotannins are effective biocides against bacteria and viruses, such as *Campylobacter jejuni, Vibrio parahaemolyticus*, and human immunodeficiency virus (HIV) (Nagayama *et al.,* 2002). Phlorotannins have also demonstrated anti-cancer, antioxidant and anti-inflammatory activities (Li *et al.,* 2011; Cardoso *et al.,* 2014; Poojary *et al.,* 2016).

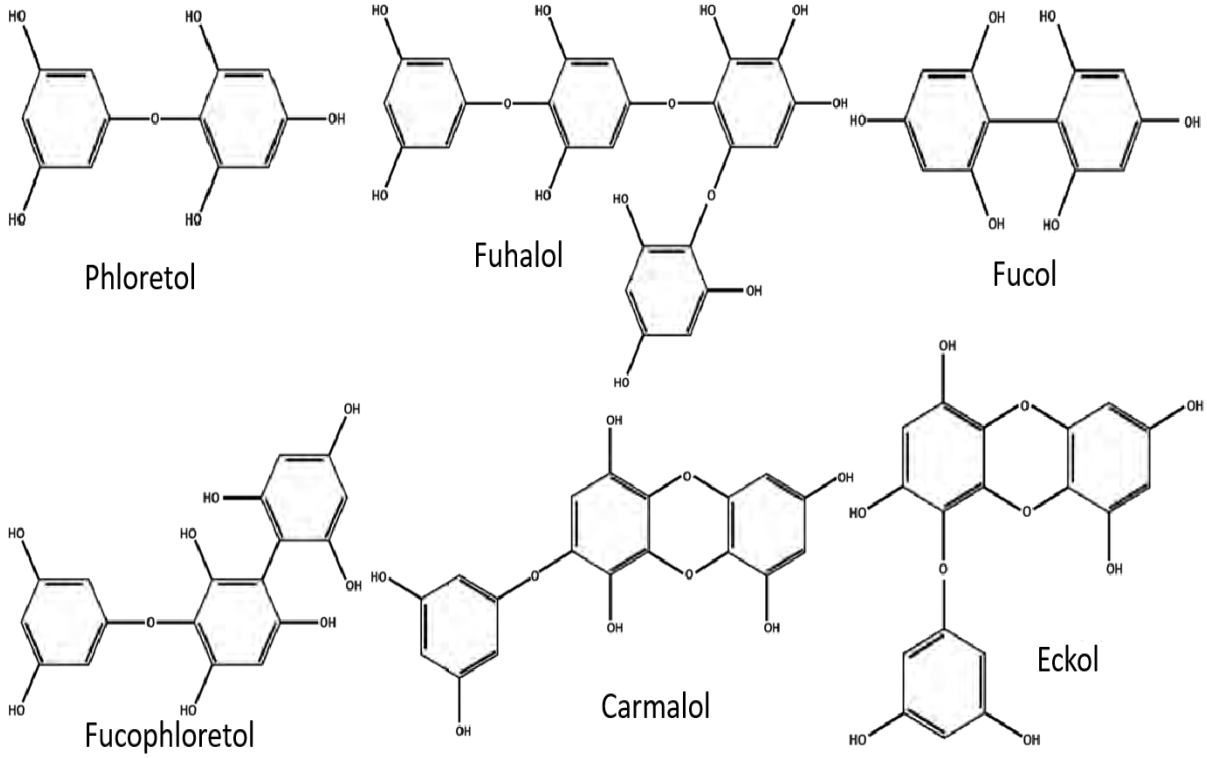


Figure 1.6: Illustrated structures of six classes of phlorotannins: phlorethol, fuhalol, fucol, fucophlorethol, eckol and carmalol.

1.3 Seaweeds in South Africa

1.3.1 Rhodophyta

Rhodophyta, known as red algae, are macroscopic, multicellular and benthic organisms usually consumed in many Asian countries (Wijesinghe and Jeon, 2011). Rhodophyta are used in many food industry applications as gelling agents, emulsifiers, and for producing phycocolloids, such as

agar and carrageenan (Anderson *et al.,* 1989; de Alencar *et al.,* 2016). In South Africa, some red algae were identified, and agar was extracted locally, due to the unavailability of imports from Japan and Britain during the Second World War (Anderson *et al.,*1989). To date, no large-scale cultivation of red algae has been conducted in South Africa. The commercial venture to cultivate red algae in the 1990s failed, and only beach casts have been milled and ground for exporting (Anderson *et al.,* 1989; Amosu *et al.,* 2013). Agar and carrageenan from red algae are extensively used in the pharmaceutical industry and molecular biology, making red algae a valuable resource (Amosu *et al.,* 2013). Recently, red algae have also been shown to provide antioxidant, antibacterial, anticoagulant and antitumor activities (de Alencar *et al.,* 2016; Khalid *et al.,* 2018). Red algae are generally found along the coast of South Africa, along the coast of False Bay, all the way to the Kwazulu-Natal coastal lines, with *Gelidium spp.* being the predominating red algae (Anderson *et al.,* 1991).

One of the most common red algal species in South Africa is *Gelidium pristoides* (Figure 1.7A). It is an endemic South African species found on the shore of Port Edward, near the border between Kwazulu-Natal and Eastern Cape, to Kommetjie, on the west coast of the Cape of Good Hope (Anderson *et al.,* 1991). From 2001 to 2010, the total yield of *Gelidium spp.* was 1,140 tonnes dry weight (Amosu *et al.,* 2013). According to Carter and Anderson (1985), the summer growth rate of *G. pristoids* takes 3 to 4 months until the preharvest biomass level, providing up to four harvests per annum. This growth rate gives *G. pristoides* a high potential for mass cultivation, especially in the Transkei region, where much seaweed farming has been conducted in South Africa.

Another red algal species in South Africa is *Gracilaria gracilis*, or *Gracilaria verrucose* (Figure 1.7B). It is often found as a beach cast in the sandy areas of the Langebaan and Saldanha Bay coastlines (Anderson *et al.,* 1989). In 1956, it was estimated that 1,000 tonnes dry weight of *Gracilaria verrucose* were washed off as beach-cast and not used. From 2001 to 2010, the total yield of *Gracillia species* was 633,089 kg dry weight (Amosu *et al.,* 2013).

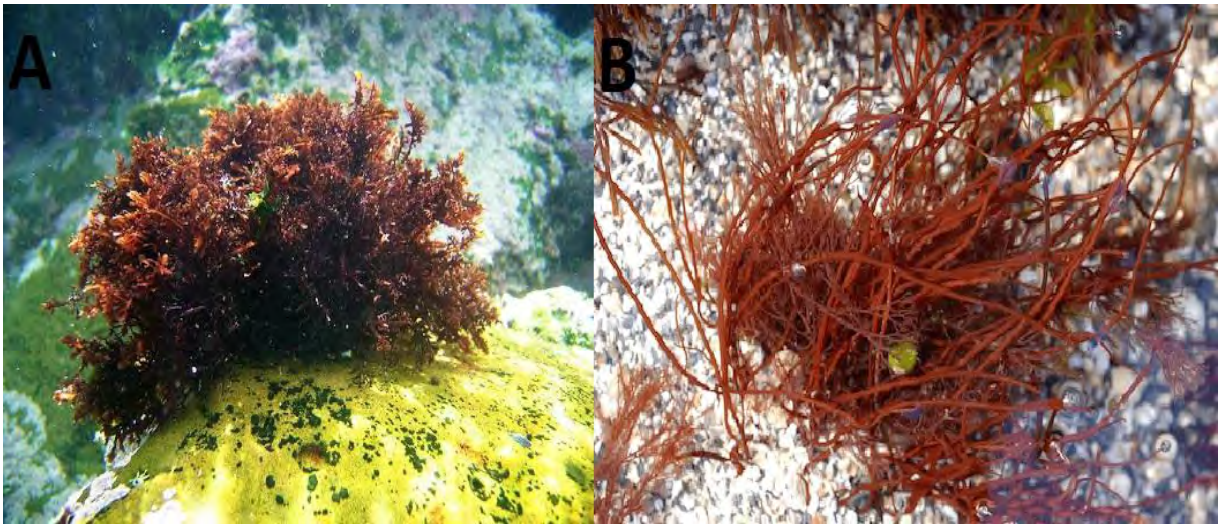


Figure 1.7: Red algae growing in the wild, **A)** *Gelidium pristoides* and **B)** *Gracilaria verrucose*. [Figures sourced from Louw, 2020; www.alchetron.com/Gracilaria]

1.3.2 Phaeophyceae

Phaeophyceae brown algae are a class of filamentous algae that range from small to large and complex seaweeds (Wehr, 2015). The distinct brown colour of brown algae comes from a carotenoid pigment, a type of fucoxanthin contained within them (Wehr, 2003). Brown algae are rare in freshwater and are typically found in saltwater as benthic organisms (Wehr, 2015). The cell walls of brown algae are composed of cellulose supplemented with the mucopolysaccharide alginic acid (Wehr, 2003). Brown algal species in freshwater generally contain less alginate than brown seaweeds in the oceans (Wehr, 2003). Brown seaweeds are farmed and cultivated worldwide for their use in numerous industries. In many East Asian countries, humans have consumed brown seaweeds for many centuries for their health benefits, notably in Korea, where a woman who has just given birth would usually consume *miyeok-guk* or seaweed soup made from *Undaria pinnatifida*, which, only recently, has been shown to have large health benefits (Hwang and Park, 2020). Recent studies have shown that *U. pinnatifida* contains large amounts of digestible protein, up to 70% (w/w) of total available protein, as well as essential vitamins and amino acids, anti-coagulant properties from a sulphated polysaccharide, fucoidan, and has been associated with antihypertensive, antioxidant, and antidiabetic effects (Admassu *et al*., 2018; Cherry *et al*., 2019).

Because most brown seaweeds contain alginate in their cell walls, alginate has been extracted for many commercial uses. It is mainly used in the food industry as a thickening agent and has also

been used in the anode of lithium-ion batteries (Kovalenko *et al.,* 2011). The bioactive compounds in brown seaweeds include alginate, fucoidans, fucoxanthin and phlorotannins (Wijesinghe and Jeon, 2011). These bioactive compounds are known for their anti-ageing, antimicrobial, anti-tumour, anticoagulant, anti-inflammatory, anti-diabetic, anti-Alzheimer and anti-malarial activities (Shannon and Abu-Ghannam, 2018; Habeebullah *et al.,* 2019; Daub *et al.,* 2020). *Ecklonia maxima* and *Laminaria pallida* belong to the class of Phaeophyceae. They are found along the cool-temperate west coast of South Africa, where they act as a surface canopy to kelp beds between Cape Agulhas and Cape Columbine (Anderson *et al.,* 2006).

Ecklonia maxima are one of the most farmed and cultivated seaweed species in South Africa (Figure 1.8A) (Ferdouse *et al.*, 2018). *E. maxima* has been collected as beach-cast and exported to many countries in Europe, North America and Asia for alginate production since the 1950s (Anderson *et al.,* 1989). Since the 1970s, *E. maxima* has been harvested by divers by cutting the seaweed just above the holdfast, which is then used to produce commercial plant-growth stimulant cocktails or regimens (Troell *et al.,* 2006). In 2011, approximately 6,000 tonnes of the wet weight of *E. maxima* was harvested for use as abalone feed, while a similar volume was harvested for the production of plant growth stimulants (Amosu *et al.,*2013; Anderson and Rothman, 2013). It is estimated that there are 1030 tonnes of *Ecklonia* fronds, the leaves of *Ecklonia*, available on the Cape Peninsula; however, only some parts can be used by the right-holder, such as Kelpak®, to prevent over-exploitation (Troell *et al.,*2006). Ferdouse *et al.* (2018) reported that the value of *E. maxima* harvested for abalone feed is estimated at ZAR 8 million per annum.

Laminaria pallida is another brown seaweed species readily found on the South African coastline (Figure 1.8B). Although it is not farmed, cultivated, or readily used compared to *E. maxima*, it is still widely available in South Africa. *L. pallida* can be found from Danger Point on the west coast to Northern Namibia (Anderson and Rothman, 2013). *Laminaria pallida* grow under *E. maxima* in deeper water, making the habitat similar to *E. maxima* (Anderson and Rothman, 2013). Data on the production and trade of *L. pallida* is not available, indicating low levels of interest or low output; however, *L. pallida* is reported to be traded to other countries where it is used in special eateries for its pleasant flavour (Ferdouse *et al.,* 2018).

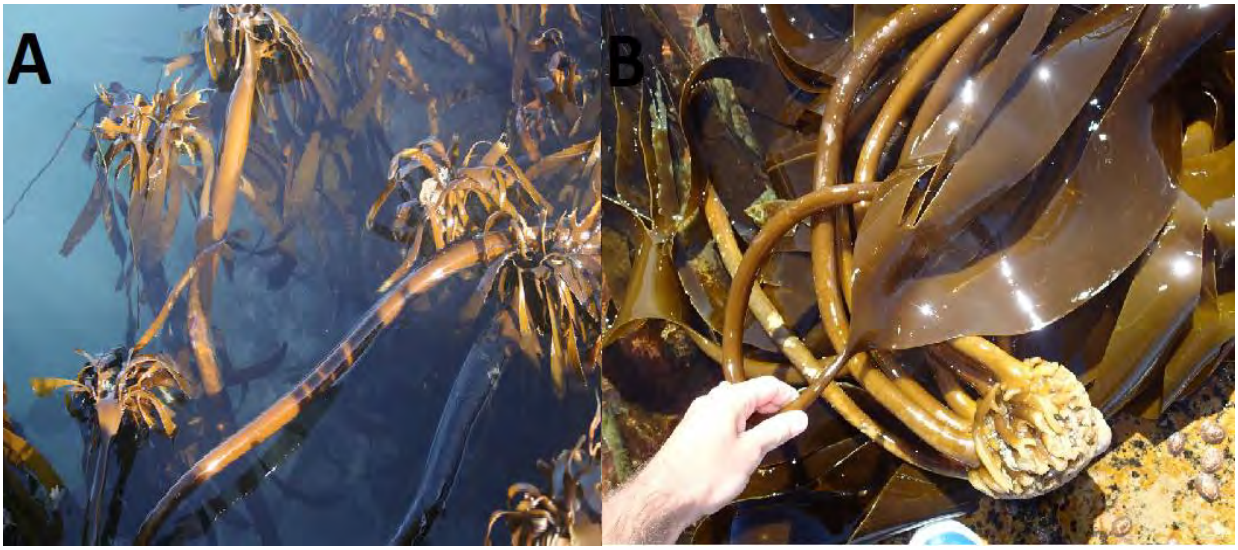


Figure 1.8: Diagrams of brown algae growing in the wild, **A)** *Ecklonia maxima* and **B)** *Laminaria pallida*. [Figures sourced from Anderson *et al.,* 2016; www.v3boldsystems.org]

1.3.3 Chlorophyta

Chlorophyta, green algae, is one of the most diverse groups of algae, containing more than 7,000 species worldwide (Algaebase.org, 2022). Like land plants, green algae have two forms of chlorophyll: a and b; therefore, they can make their food and absorb nutrients from water through their cells (Roleda and Hurd, 2019). However, they are primarily aquatic and lack vascular systems and roots; thus, they are called "*algae*" (Pal *et al.,* 2014). Green algae can be found in both fresh and saltwater. Green algae are not commonly used as sea vegetables; unlike red and brown algae, however, it has recently been reported that Ulvales are used as food condiments and nutritional supplements in China, the United States of America (USA), Japan, France and Chile (Nagappan and Vairappan, 2013).

Green algae have also been used to treat multiple diseases and improve human health (Fu *et al.,* 2017). A homogeneous sulphated heterorhamnan from green algae, *Gayralia oxysperma*, has shown anti-viral activity against the herpes simplex virus (Cassolato *et al.,* 2008). Astaxanthin, traditionally used to produce pigments, has also been reported to have high antioxidant properties and is thus used in nutritional supplements (Li *et al.,* 2015). Many carotenoid substances can also be found in green seaweeds, such as lutein, β-carotene and fucoxanthin (Pérez *et al.,* 2016). The most notable green seaweed in South Africa is the genus *Ulva* (Figure 1.9A). Green seaweeds can

be found on the west coast; however, the harvest has been delayed due to administrative reasons (Anderson and Rothman, 2013). Humans consume *Ulva* in many Asian countries; however, *Ulva* is mainly produced in South Africa as abalone feed in ponds and raceways with an aeration system in the Eastern Cape (Figure 1.9B). In 2013, it was reported that 2,015 tonnes of wet weight were harvested for abalone feed alone in South Africa (Ferdouse *et al.,* 2018).

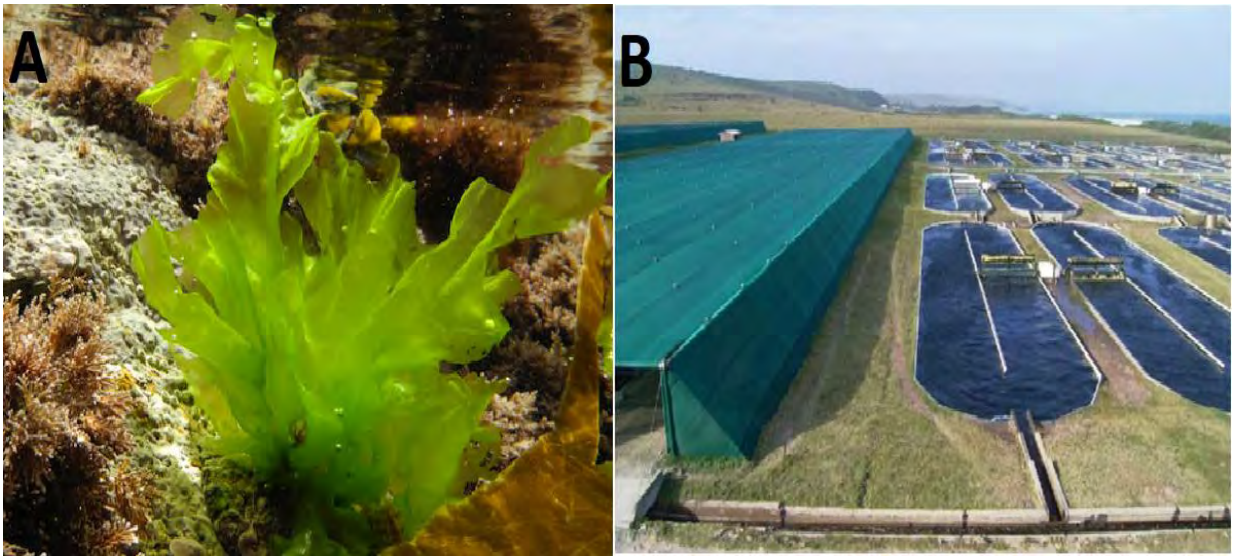


Figure 1.9. **A)** *Ulva lactuca* growing in the wild and **B)** seaweed farm in Eastern Cape with raceways. [Figures are sourced from www. seaweed.ie; Anderson and Rothman, 2013]

1.4 Extraction methods

1.4.1 Conventional solvent extraction processes

Traditionally, the extraction of algal intercellular products has been conducted with organic or aqueous solvents (solvent extraction processes), depending on the polarity of the target compounds (Lopes *et al.,* 2016). For example, carotenoids have varying polarities, solubilities and chemical stabilities; a suitable solvent must be selected for a specific compound, allowing extraction with greater efficiency and purity (Poojary *et al.,* 2016). The solvents used for the solvent extraction include acetone, octane, ethanol, hexane and dimethyl sulphoxide. However, these solvents require many steps to extract compounds, harming the environment (Maki-Arvela *et al.,* 2014; Shannon and Abu-Ghannam, 2018). Therefore, greener extraction technologies have recently been receiving more attention. These extraction technologies include greener solvent extraction, microwave-

assisted extraction (MAE), enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE) and the cell burst method (Poojary *et al.,* 2016; Deenu *et al.,* 2013).

Traditionally, conventional extraction of compounds from brown seaweeds involves multi-stage processing, which includes cleaning/washing, pre-treatment, solid/liquid separation, precipitation and filtration, and drying and milling of the biomass (Hernández-Carmona *et al.,* 2013; Abdul Khalil *et al.,* 2018). Involving a multi-stage process makes extraction tricky as the compounds are affected by different chemicals at various stages. Moreover, this method requires a lot of chemicals, water and energy, generating a lot of waste in the process, which is environmentally unfriendly and costly (Hernández-Carmona *et al.,* 2013). Typically, the conventional solvent extraction process extracts hydrocolloids, such as carrageenan and alginate, by exploiting their water solubility (Abdul Khalil *et al.,* 2018). Hot water is usually used to extract these compounds and utilises acidic and alkaline solutions as major solvents (Rhein-Knudsen *et al.,* 2015; Hernández-Carmona *et al.,* 2013).

The main drawbacks of current conventional extractions are the vast water consumption, time, and energy requirement compared to greener extraction methods (Dey and Rathod, 2012; Rodrigues *et al.,* 2015). For example, with the conventional solvent extraction methods, the duration for extracting alginate or agar is approximately 2 – 4 hours. Still, greener methods could reduce it to a few minutes (Abdul Khalil *et al.,* 2018). However, there are more significant limitations with greener extraction methods, such as very high capital cost, the lack of efficient enzymes, knowledge gap, low selectivity of compounds (polar and non-polar) and lack of scale-up data (Heng *et al.,* 2013; Grosso *et al.,* 2015; Abdul Khalil *et al.,* 2018). Most greener extraction methods are still underdeveloped and there is a lack of published knowledge compared to conventional solvent extraction procedures, making the solvent extraction method more favourable and desirable (Abdul Khalil *et al.,* 2018).

1.4.2 Microwave-assisted extraction processes

The MAE process uses non-ionising electromagnetic radiation with a frequency that ranges from 300 MHz to 300 GHz. This radiation transfers heat into the cells through dipole rotation of molecules and ionic conduction in the medium (Chémat and Cravotto, 2013). The heat transfer by the microwave can heat the cells, which causes pressure to build up inside the cells (Poojary *et al.,*

2016). This pressure can rupture the cells and increase their porosity, which allows easier penetration of solvents to extract compounds within the cells (Poojary *et al.,* 2016). The MAE process has two reaction vessel options: open and closed vessels. Open vessels are conducted under low temperatures at atmospheric pressure, while closed vessel systems operate at high temperatures (Chémat and Cravotto, 2013).

The MAE process has often been used to extract bioactive compounds from seaweeds. Xiao *et al.* (2012) used the process to extract fucoxanthin from three edible brown seaweeds within 75 minutes. Their study also concluded that fucoxanthin was relatively stable, unaffected by the microwave's energy level. Esquivel-Hernández *et al.* (2016) reported that they could isolate carotenoids from *Arthrospira platensis* using MAE at 400 W, 50°C and a pressure of 1 bar. Ruen-ngam *et al.* (2010) extracted astaxanthin from *Haematococcus pluvialis* using MAE, obtaining a 74 ± 4% (w/w) yield at 75°C for 5 minutes. However, a temperature over 75°C resulted in a loss in the recovery of astaxanthin. This was due to carotenoids being very sensitive to temperature, leading to their decomposition (Poojary and Passamonti*,* 2015a; Poojary and Passamonti*,* 2015b). MAE is a rapid and greener method for extracting bioactive compounds, but microwave power and temperature must be controlled accurately, as it can lead to the degradation of compounds (Poojary *et al.,* 2016).

1.4.3 Ultrasound-assisted extraction processes

The UAE is a novel, inexpensive procedure compared to other greener extraction methods (Deenu *et al.,* 2013). The UAE process is a replacement for the solvent extraction processes, which uses many organic solvents, such as ethanol, acetone, hexane and dimethyl sulfoxide, which need to be discarded after use and cause harm to the environment (Shannon and Abu-Ghannam, 2018). The solvent extraction method requires many steps, making it time-consuming and causing the loss of compounds during different stages (Maki-Arvela *et al.,* 2014). Therefore, UAE is one of the ideal methods for extracting bioactive compounds from seaweeds with short extraction times and fewer solvents (Abdul Khalil *et al.,* 2017). UAE utilises acoustic cavitation to produce cavitation bubbles that implode, resulting in a high shear force. The implosion disrupts cell walls, allowing the solvent to penetrate the cells for better recovery of bioactive compounds (Vinatoru *et al.,* 1997). An advantage of the UAE is its flexible frequency range, from 20 kHz to 4 MHz (Leong *et al.,* 2013).

This allows researchers to select the desired acoustic frequency of ultrasound for different specimens.

A study by Deenu *et al.* (2013) showed that lutein, a type of carotenoid, could be extracted up to 3.16 ± 0.03 mg/g wet *Chlorella vulgaris* within 5 hours using UAE at 35 kHz. In another study by Zou *et al.* (2013), astaxanthin, a type of carotenoid, was extracted up to 27.58 ± 0.4 mg/g from *Haematococcus pluvialis* within 16 minutes using UAE at 40 kHz. These studies show how the efficiency and flexibility of the UAE are ideal for extracting various compounds. Overall, UAE provides increased extraction yields and rates and reduced extraction times compared to conventional extraction methods.

1.4.4 Enzyme-assisted extraction processes

Seaweed cell walls comprise highly complex compounds, including polysaccharides such as alginate, fucoidan, laminarin, cellulose, pectin and hemicellulose, and lignin in macroalgae (Kim, 2012). These polysaccharides are known to be challenging to degrade due to their insolubility in different solvents, as they are present as hydrogen-bonded crystalline fibres in seaweed cell walls (Kim, 2012). This makes it difficult for solvents to access the bio-compounds of interest during extraction (Fleurence *et al.,* 1995; Doi and Kosugi, 2004). In recent years, the enzyme-assisted extraction (EAE) process for seaweeds has been shown to improve the extraction efficiency of bioactive compounds (Hardouin *et al*., 2014; Puspita *et al.*, 2017; Vasquez *et al.,* 2019). Carbohydrate-Active enzymes and proteases have been used to study the degradation of cell walls of seaweeds to help release a variety of bioactive compounds (Heo *et al.,* 2005; Habeebullah *et al.,* 2019). Enzymes have also been used to convert water-insoluble compounds to water-soluble compounds, which is advantageous as a green method for extracting bioactive compounds compared to the traditional solvent extraction procedures (Habeebullah *et al.,* 2019). Many enzymes can be utilised for the extraction process; however, due to cell wall and bioactive compound composition diversity among seaweeds, it is essential and often challenging to choose the correct enzyme(s) for this process (Wijesinghe and Jeon, 2012). Furthermore, the optimum pH and temperature of the enzymes must be maintained during the extraction process (Wijesinghe and Jeon, 2012). A study by Shannon and Abu-Ghannam (2018) showed a fucoxanthin yield of 93.56% from the blade and 107.96% from the holdfast, using Viscozyme® L, which degrades the cell walls

of the seaweed, during the acetone extraction process. The EAE process has also resulted in higher extraction yields and 50% better radical scavenging biological activity of extracted compounds than the solvent extraction process (Heo *et al.,* 2003).

1.4.5 Cell burst method

The cell bursting method has been traditionally used to burst bacterial and fungi cells using a homogeniser or a French press to obtain intracellular materials. Kelpak® has developed a cold cellular-burst technology for seaweed and has patented the method that has been extensively used to obtain seaweed concentrate as a plant bio-stimulant (Stirk *et al.,* 2004; Cellburst™ – Kelp Products International (Pty) Ltd. 2018). Seaweed concentrate is mainly obtained from *E. maxima* and generally contains amino acids, vitamins, auxins and other mineral compounds (Stirk *et al.,* 2004; Moncada *et al.,* 2022). Seaweed biomass is cut into fine particles and passed through high pressure and then under low pressure at high velocity. The change in pressure expands the cell walls, exceeding the elastic limit of the cell; this results in the rupture of the cells and the release of internal components (Poojary *et al.,* 2016). The cell bursting method does not utilise heat, chemicals, solvents or dehydration, resulting in no degradation of the seaweed components. This makes the cell burst method environmentally friendly and safe as a bio-stimulant production protocol (Stirk *et al.,* 2004). Kelpak® has used this method for the past four decades to produce an effective bio-stimulant for application on vegetables, flowering plants, trees and monocotyledonous crops (Metting *et al.,* 1990).

1.5 Chapter conclusion

Seaweeds have been utilised for centuries, particularly in Asian countries, for their health benefits and diverse applications. However, in South Africa, their potential remains largely untapped despite the country's access to abundant species of seaweed. Seaweeds are categorised into Rhodophyta (red algae), Phaeophyceae (brown algae), and Chlorophyta (green algae), each characterised by unique chemical properties that contribute to their diverse applications. This chapter highlighted the role of seaweeds in various industries, including pharmaceuticals, nutraceuticals, cosmetics, and food production. These applications are largely driven by the bioactive compounds found in seaweeds, such as hydrocolloids (agar, carrageenan, alginate),

polysaccharides (fucoidan), carotenoids (fucoxanthin), and polyphenols (phlorotannins). These compounds exhibit various bioactivities, including anti-inflammatory, antioxidant, anti-viral, anti-diabetic, and anti-cancer activities.

In South Africa, seaweed farming and harvesting have been primarily focused on producing abalone feed and agricultural bio-stimulants, with limited efforts directed towards higher-value applications. Among the commercially significant species are *Ecklonia maxima* and *Laminaria pallida* (brown algae), and *Gelidium pristoides* and *Gracilaria verrucose* (red algae). Despite the challenges of large-scale cultivation, these species demonstrate significant potential for expansion into industries such as pharmaceuticals and nutraceuticals. While South Africa accounts for only a small percentage of the global seaweed harvest, it has an opportunity to capitalise on its marine biodiversity.

In this chapter, the importance of extraction technologies in unlocking the full potential of seaweed-derived products was also discussed. Traditional solvent extraction methods, while widely used, are associated with high energy and water consumption, and environmental impact. As an alternative, greener extraction technologies, including microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), and the cell burst method, offer significant advantages such as reduced processing times and improved yields. Although greener methods are still under development and face challenges such as high capital costs and limited scalability, they represent a promising direction for sustainable seaweed processing.

Seaweeds are valuable due to their diverse bioactive compounds and ecological benefits. Hydrocolloids such as agar, carrageenan, and alginate are widely used in foods and pharmaceuticals, while polysaccharides (like fucoidan) and carotenoids (like fucoxanthin) show therapeutic potential. Phlorotannins, unique to brown algae, provide antimicrobial, antioxidant, and anti-inflammatory benefits. Seaweeds can, therefore, play a significant role in addressing global health challenges.

Despite the significant potential of South African seaweeds, the industry is hindered by a lack of expertise and infrastructure to extract and commercialise bioactive compounds efficiently. By

advancing research in developing sustainable extraction technologies, and fostering partnerships between academic institutions, industry players, and government, South Africa can unlock the full economic and ecological value of its seaweed resources.

In conclusion, South Africa has an opportunity to position itself as a global leader in the seaweed industry by diversifying applications beyond abalone feed and agricultural stimulants. The integration of greener extraction technologies and the expansion of research into studying the bioactive potential of local species, such as *Ecklonia maxima* and *Gelidium pristoides*, could drive the development of high-value seaweed-derived products. Furthermore, increasing awareness and investment in this field can contribute to job creation, environmental sustainability, and economic growth. With the global demand for sustainable natural resources continuing to rise, South Africa's seaweed industry holds great promise for addressing both local and global challenges while fostering a vibrant blue economy.

Chapter 2 – Research Motivation and Hypothesis

2.1 Problem statement

Extraction technologies for fucoidan and sodium alginate have been studied extensively over the last decade to obtain these compounds for commercial use. Brown seaweeds remain an attractive source of fucoidan and sodium alginate, as these polysaccharides account for up to 40 – 47% of the dry weight of seaweeds (Cho *et al.,* 2010; Fitton *et al.,* 2015; Holdt and Kraan, 2011). Brown seaweed fucoidans have various bioactive properties, such as antioxidant, anticoagulant, anticancer, anti-inflammatory, antiviral, antioxidant and anti-diabetic activities (Kumar *et al.,* 2014; Li *et al.,* 2008). Sodium alginates from brown seaweeds have also demonstrated bioactivity properties, including anti-obesity and anti-diabetic properties (Wan-Loy and Siew-Moi, 2016). Alginates and alginates in other forms, such as sodium alginate or calcium alginate, are used for their gelling properties in cosmetics, pharmaceuticals and food (Holdt and Kraan, 2011; Tseng, 2001). Therefore, researchers have been studying and developing methods to extract fucoidan and sodium alginate, which should, ideally, yield the most quantities at high purity with the least effort and economic cost (Holdt and Kraan 2011).

Due to these reasons, many extraction technologies, such as ultrasound-assisted, enzyme-assisted and microwave-assisted extractions, have been studied extensively to obtain bioactive compounds from seaweeds (Habeebullah *et al.,* 2019; Jacobsen *et al.,* 2019; Juliano *et al.,* 2017). However, these extraction technologies are limited in terms of the cost and scale of the extraction procedure compared to conventional solvent extraction methods, such as acidic treatment and green solvent methods (Poojary *et al*., 2016). Traditional extraction procedures utilise polar and non-polar solvents, such as *n*-hexane, dichloromethane, dimethyl ether and diethyl ether, and organic solvents, such as acetone, octane and ethanol, which affords them a significant advantage over other extraction technologies when scaling up and costing of the overall process and equipment required (Poojary *et al.,* 2016).

Due to the challenges of non-conventional extraction methods, many studies have attempted to obtain the maximum fucoidan and sodium alginate yields in one consolidated process that is less expensive and can be scaled up easily. Lorbeer *et al.* (2015) achieved a 3.21% (dry weight) yield for fucoidan and a 45.5% (dry weight) yield for sodium alginate using conventional solvent

extraction from *Ecklonia radiata* beach cast from south Australia. In another study by Daub *et al.* (2020), a 6.89% (dry weight) fucoidan yield was achieved using hot water extraction from *Ecklonia maxima* in South Africa. A recent study by Darko et al. (2024) achieved 11.1% (dw) of sodium alginate from *E. maxima* from Saldana Bay, South Africa, using 0.05 M HCl at 80°C. However, these studies only utilised 7.5 and 20 g of seaweed, with the feasibility of scaling up the conventional extraction process not being investigated. Greener extraction methods are more beneficial to the environment and can be efficient; however, at this point, the required equipment is expensive and requires more technical knowledge to be used efficiently. Therefore, conventional solvent extraction must be optimised to extract valuable seaweed compounds. The potential to obtain large amounts of fucoidan and sodium alginate from South African brown seaweeds has not been fully explored. Therefore, this study will address the knowledge gap and technological shortcomings by optimising the sequential extraction of the fucoidan and sodium alginate contents of South African brown seaweeds. The optimisation of fucoidan and sodium alginate extraction will focus on the pH and temperature of the solution during extraction and the final ethanol concentrations of the solution during the precipitation of fucoidan and sodium alginate.

2.2 Hypothesis

Fucoidan and sodium alginate can be extracted sequentially from South African brown seaweed, *Ecklonia maxima*. This sequential extraction process can be optimised to provide higher yields than the single compound extraction process while achieving products with suitable characteristics for commercial use.

2.3 Aims and Objectives

- To optimise the sequential extraction of fucoidan and sodium alginate from a South African brown seaweed, *Ecklonia maxima*;
- To chemically and structurally characterise the extracted compounds;
- To scale up the extraction process more than ten-fold and compare the results with that obtained with small-scale lab extraction; and
- To recover the ethanol used in the extraction for a greener extraction method.

Chapter 3 – Materials and Methods

3.1 Commercial fucoidan and sodium alginate

Fucoidan from *Fucus vesiculosus* (Cat No. F5631) and sodium alginate (Cat No. W201502) were purchased from Sigma-Aldrich (St Louis, MO, USA). All other reagents and assay kits were of analytical grade and purchased from Sigma-Aldrich, MERCK (Darmstadt, HE, Germany) and Megazyme™ (Bray, WC, Ireland).

3.2 *Ecklonia maxima* biomass

Mixed thalli of *Ecklonia maxima* were supplied by KelpX (Pty) Ltd, harvested from Port Nolloth (-29.253997, 16.867888), South Africa, which were minced and sundried. After collection, the seaweed was cut into small pieces (4-5 cm) before being ground into powder using a Platinum® coffee grinder (Model: JC-CG150W, Johannesburg, South Africa). The obtained powder was stored in a dark, dry place until further use.

3.3 Sequential extraction process

Fucoidan and sodium alginate were extracted according to a modified sequential biorefinery extraction process described by Lorbeer *et al.* (2015) (Figure 3.1). Ground seaweed, Biomass A, was depigmented in ethanol twice for 2 hours each. Depigmented seaweed, Biomass B, was dried at room temperature for 24 hours. Seaweed (Biomass B) was placed into a pH 1.0 HCl (0.1 M) solution of 30× the seaweed's dry weight and left for 2 hours at room temperature with constant stirring. Extract A was concentrated with 99% ethanol to a final ethanol concentration of 80% for fucoidan extraction and was left overnight at room temperature. The precipitated fucoidan was filtered over a cheesecloth, and the extracted fucoidan was dried at room temperature overnight. Biomass C (Figure 3.1), residual biomass that contained sodium alginate, was added to a 1 M Na_2CO_3 solution in a solid-to-liquid ratio of 1:50 and left at room temperature overnight with constant stirring at 100 rpm. A cheesecloth was then filtered to separate Biomass D and Extract B. Extract B was adjusted to a final ethanol concentration of 67% and left for 1 hour to precipitate sodium alginate. Sodium alginate was squeezed by hand and washed with methanol and acetone, 50 ml each, for 1 minute before drying at room temperature overnight. Dry sodium alginate was

washed by redissolving it in dH_2O in a solid-to-liquid ratio of 1:50 of the original weight and left to stir overnight before adjusting it to a final ethanol concentration of 67% (v/v). Washed sodium alginate was treated in a similar manner as mentioned above with methanol and acetone.

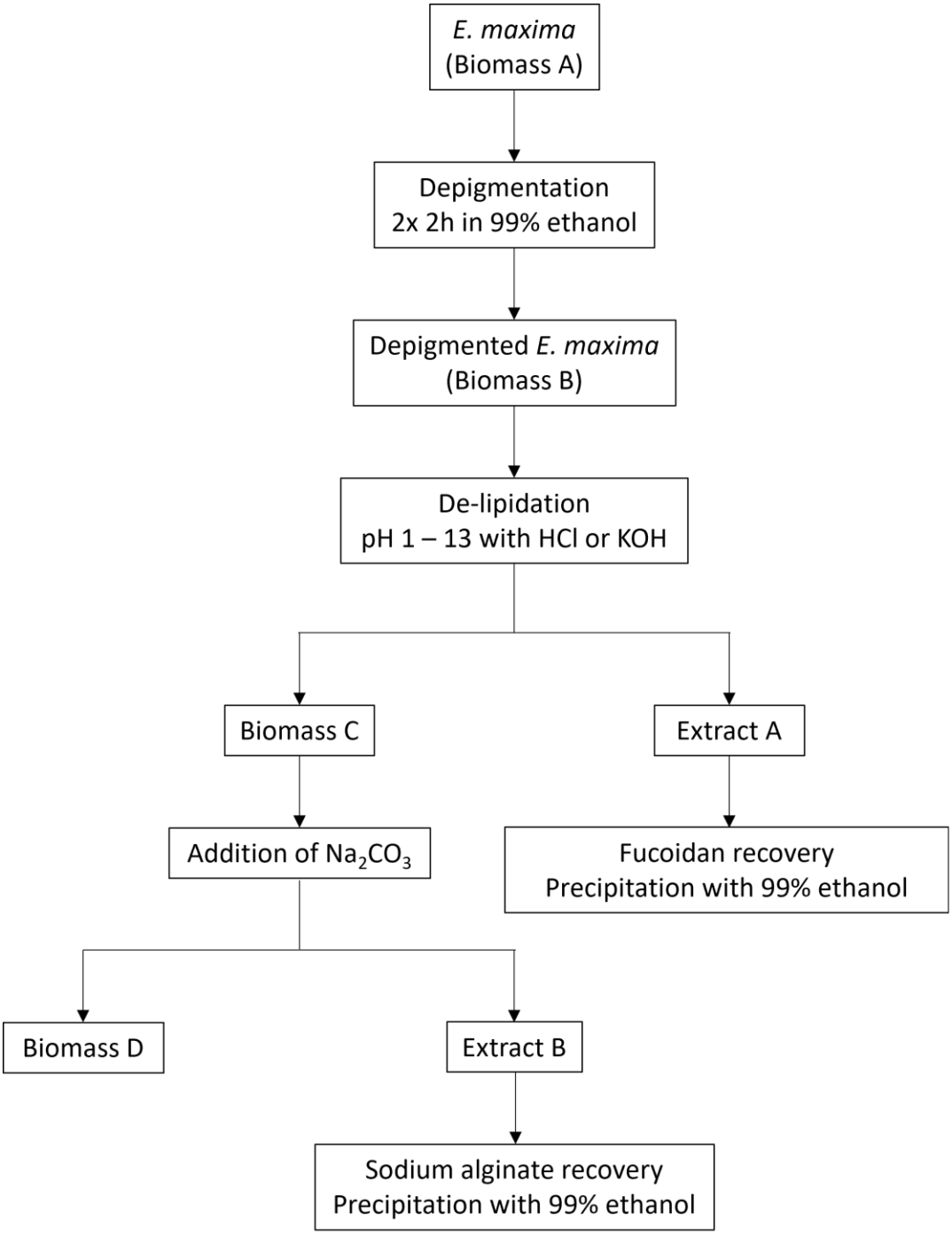


Figure 3.1: Scheme indicating the workflow of the sequential extraction of fucoidan and sodium alginate.

3.4 Compound Extraction

3.4.1 Changing variables in order to optimise compound extraction

Variables in each step of the extraction of fucoidan and sodium alginate were changed in an attempt to improve the product yield. The extracts obtained from the changed variables were characterised to compare them to each other and commercially available compounds. Only one variable was changed at each step, and the optimal conditions at each step were combined to establish the final overall extraction protocol.

3.4.2 Depigmentation

The depigmentation step was performed by washing the seaweed with 99% (v/v) ethanol in a solid-to-liquid ratio of 1:20 for 24 hours. The depigmented seaweed biomass was dried at room temperature and kept in an airtight bag until further use.

3.4.3 Delipidation (Acid and alkaline treatment)

The delipidation step was optimised by varying the pH during the extraction process described by Lorbeer *et al*. (2015). The different pH solutions were made by adjusting the pH with either 2 M HCl or KOH, and 7.5 g of *E. maxima* was used for each test; KOH was used for the alkaline solution instead of NaOH to prevent sodium alginate forming during the fucoidan extraction step. The pH solutions of 30× the seaweed weight were used for the delipidation step and left stirring for 2 hours at 100 rpm before decanting Extract A (Figure 3.1). Extract A was filtered through cheesecloth to remove any solids. Fucoidan was precipitated by adjusting the final ethanol concentration of Extract A to 80% (v/v) at room temperature overnight. Sodium alginate was extracted by treating Biomass C with 1 M Na_2CO_3 with a volume of 50× the initial seaweed weight, with constant stirring overnight at a room temperature of 22°C. Extract B (Figure 3.1) was decanted and filtered through cheesecloth to remove any solids. Sodium alginate was extracted by adjusting the final ethanol concentration of Extract B to 67% (v/v). The yields of sodium alginate and fucoidan were compared after each variation of the protocol. The protocol that produced the highest extract yields was selected for subsequent steps of the study.

3.4.4 Variation in temperature during the delipidation of seaweed

The best yield from the delipidation procedure detailed in section 3.4.3 was repeated at 10°C, 22°C and 37°C. The incubation temperature that resulted in the highest yield of fucoidan was selected for subsequent studies.

3.4.5 Effect of ethanol concentration on fucoidan extraction

The optimum final ethanol concentration required to extract fucoidan was optimised using the optimal conditions established in section 3.4.4. The optimisation was performed by adding 99% ethanol to Extract A, which was then diluted to range from 50% to 90% (v/v) in increments of 10%. Extract A was first adjusted to a final ethanol concentration of 50% and left overnight at room temperature. It was filtered through cheesecloth to collect fucoidan while collecting the solution for further ethanol concentration study. Extract A, at 50% final ethanol concentration, was adjusted to 60% ethanol concentration and left overnight at room temperature. It was filtered through cheesecloth to collect any fucoidan while collecting the solution. This was performed for every 10% final ethanol concentration increment until the final ethanol concentration of 90% was reached.

3.4.6 Effect of Na_2CO_3 concentration on alginate extraction

The optimal concentration of Na_2CO_3 required for biomass C (Figure 3.1) was determined by varying the concentration of Na_2CO_3 from 0 M to 3 M in increments of 0.5 M (unwashed). The extraction was repeated, and one batch was re-solubilised in dH_2O to 5% (w/v) and re-extracted to wash any residual sodium carbonate (washed). Thermal gravimetric analysis (TGA) was used to test the unwashed and washed sodium alginates for ash content. TGA was conducted with a PerkinElmer® (Pyris Diamond model) thermogravimetric analyser (Waltham, USA) following the method of Mabate et al. (2021). Sodium alginate extracted using the highest sodium carbonate concentration, which exhibited minimal differences in ash content compared to its washed counterpart, was selected for further studies.

3.4.7 Effect of ethanol concentration on alginate extraction

The optimum final ethanol concentration required to extract sodium alginate was established using the best result from 3.4.6. The optimum final ethanol concentration required to extract sodium alginate was determined by adding 99% ethanol to Extract B to an adjusted final ethanol concentration ranging from 50% to 90% in increments of 10%. Extract B was first adjusted to a final ethanol concentration of 50% and left overnight at room temperature. It was filtered through cheesecloth to collect sodium alginate while collecting the solution. Extract B, at 50% final ethanol concentration, was adjusted to 60% final ethanol concentration and left overnight at room temperature. Again, it was filtered through cheesecloth to collect any sodium alginate while collecting the solution. This was performed for every 10% final ethanol concentration increment until a final concentration of 90% was reached.

3.4.8 Washing of sodium alginate

Sodium alginate was washed by dissolving all the extracted sodium alginate in dH_2O, in a volume of 50× of the initial weight of seaweed, with constant stirring overnight. Sodium alginate was precipitated using the optimal final ethanol concentration established in section 3.4.7 and dried.

3.5 Optimised sequential extraction process for large-scale

Fucoidan and sodium alginate were extracted according to the optimised conditions established in this chapter. Ground seaweed, Biomass A (50, 100, 150, 250 g), was depigmented in ethanol twice for 2 hours each. The depigmented seaweed, Biomass B, was dried at room temperature for 24 hours. Biomass B was transferred into a pH 1.0 HCl (0.1 M) solution of 30× the mass of Biomass B and left to stir continuously for 2 hours at room temperature at 100 rpm. Extract A was then concentrated with 99% ethanol to a final ethanol concentration of 80% (v/v) (for fucoidan extraction) and was left overnight at room temperature. The precipitated fucoidan was filtered through cheesecloth, and the extracted fucoidan was allowed to dry at room temperature overnight. The remaining biomass, Biomass C, was transferred into a 0.5 M sodium carbonate solution of 50× Biomass B's mass and left at room temperature overnight with constant stirring at 100 rpm. Biomass D and Extract B were then separated using filtration through cheesecloth. Extract B was concentrated with 99% ethanol to a final ethanol concentration of 70% (v/v) and left for 1 hour to

precipitate the sodium alginate. The extracted sodium alginate was squeezed by hand and washed by dipping and vigorously shaking the extract in 100 ml each of methanol and acetone before drying at room temperature.

3.6 Analysis of extract purity

3.6.1 Protein content determination

The Bradford assay (Bradford, 1976) was used to determine the protein content of the fucoidan and sodium alginate extracts. A volume of 25 µl of blank (dH_2O) or extracts (1 mg/ml) was aliquoted into wells of a 96-well microtiter plate, and 230 µl of Bradford Reagent was added. Samples were incubated at room temperature for 20 minutes, and absorbance readings were taken at 595 nm using a PowerWaveX™ spectrophotometer (Bio-Tek Instruments Inc., Winooski, VT, USA) with KC Junior software. Protein concentrations were calculated using a protein standard curve constructed using varying concentrations (0 – 0.6 mg/ml) of bovine serum albumin (BSA) (Appendix B.1).

3.6.2 Phenolic content determination

The total phenolic content in the extracts was determined as described by Malgas *et al.* (2016) using the Folin-Ciocalteu method. To a 96-well microtiter plate, 10 µl of extract (1 mg/ml) was added with 180 µl of dH_2O and 20 µl of Folin-Ciocalteu reagent. These reagents were mixed and allowed to stand for 10 minutes at room temperature. Following this, 50 µl of 2 M sodium carbonate was added to each well and incubated for 30 minutes at 37˚C. ccccc. The total phenolic content was calculated using a phenolic standard curve constructed using varying stock concentrations of gallic acid (0 – 1 mg/ml) (Appendix B.2). The results were expressed as % of gallic acid equivalents per g dry weight.

3.6.3 Fucoidan sulphate content determination

The extracted fucoidan was made up to 2 mg/ml with 60% (v/v) formic acid and then hydrolysed overnight at 100˚C. Following hydrolysis, the fucoidan sample was cooled down for an hour and dried using a Centrivap (Vacutec Ltd., Roodepoort, South Africa) for 6 hours at 80˚C. The sample

was reconstituted to 2 mg/ml with dH_2O and mixed thoroughly. The gelatine-barium method described by Dodgson *et al*. (1961) was used to determine the sulphate content. The gelatin solution was prepared in 100 ml of hot water (60-70°C) by dissolving 0.5 g of gelatin and then storing the solution at 4°C overnight. Two grams of $BaCl_2$ were dissolved in the gelatin solution and allowed to stand for three hours at 25°C. Approximately 0.2 ml of hydrolysed fucoidan (2 mg/ml) was added to 3.8 ml of 0.5 M HCl and 1 ml of $BaCl_2$-gelatin reagent, and the mixture was allowed to stand for 20 min. A blank was prepared with 0.2 ml of water. The sulphate content was calculated from a gelatine-barium standard curve constructed using varying sodium sulphate concentrations (0 – 1 mg/ml). The absorbance readings were taken at 360 nm using a PowerWaveX™ spectrophotometer with KC Junior software (Appendix B.3).

3.6.4 Fucoidan and sodium alginate monosaccharide composition analysis

An (extracted) 20 mg/ml fucoidan solution was prepared with 2 M trifluoroacetic acid (TFA) and hydrolysed for 8 hours at 100˚C. Following hydrolysis, the fucoidan sample was cooled and dried in a Centrivap (Vacutec Ltd., Roodepoort, South Africa) for 6 hours at 80˚C. The sample was reconstituted to 20 mg/ml with dH_2O and mixed well. The D-glucose content of the fucoidan sample was quantified using the manufacturer's glucose oxidase/peroxidase (GOPOD) method. The D-glucose content was calculated from the glucose standard curve constructed using glucose as a standard (0.1 – 1 mg/ml). The absorbance readings were taken at 510 nm using a PowerWaveX™ spectrophotometer with KC Junior software (Appendix B.4). The quantification of D-xylose, D-galactose, D-glucuronic acid, L-fucose, D-fructose and D-mannose was performed as described in the Megazyme^{TM} kits (K-XYLOSE, K-LACGAR, K-URONIC, K-FUCOSE, and K-MANGL).

3.6.5 Total sugar determination for fucoidan and sodium alginate extracts

The extracts' total sugar content, with free reducing groups, was determined by performing the phenol-sulfuric acid method described by Dubois *et al.* (1956). The samples were made to 1 mg/ml using dH_2O, and 100 µl of this sample was added to 300 µl of concentrated sulfuric acid, followed by 50 µl of 5% (w/v) phenol. The samples were mixed and incubated at 90˚C for 10 minutes and then cooled to room temperature. The 200 µl samples were then transferred to a 96-well microtiter

plate, and the absorbance readings were taken at 490 nm using a PowerWaveX™ spectrophotometer with KC Junior software. The total sugar content of fucoidan was calculated from the phenol-sulfuric standard curve constructed using varying concentrations of L-fucose (0.1 – 1 mg/ml) (Appendix B.5.1). The same procedure was performed with the extracted sodium alginate samples. The total sugar content of sodium alginate was calculated from the phenol-sulfuric standard curve using varying concentrations of commercial sodium alginate as a standard (Appendix B.5.2).

3.6.6 FTIR characterisation

Fourier transform infrared spectrometer (FT-IR) analysis was performed using a Spectrum 100 FT-IR spectrometer system (Perkin Elmer, Wellesley, MA, USA) with Spectrum™ One software to characterise the fucoidan and sodium alginate samples. Baseline and ATR correction for penetration depth and frequency variations were carried out using this program. The samples were pressed uniformly and tightly against the spring-loaded anvil, and the spectra were recorded from 4000 to 600 cm^{-1}.

3.6.7 NMR characterisation

The sodium alginate extracts (1 mg/ml) were dissolved in D_2O, followed by mixing. The deuterium-exchanged samples were subjected to ^{1}H-NMR analysis. Spectra were recorded at 70°C using a Bruker 400 MHz spectrometer (Billerica, Massachusetts, USA), and analysed with Mnova 15.0.1 software (Mestrelab Research, Santiago de Compostela, A Coruria, Spain). The chemical shifts were expressed in ppm relative to tetramethylsilane (TMS) or the residual solvent signals.

3.6.8 Circular dichroism spectroscopy

Sodium alginate solution of 0.25 mg/ml was scanned using Chirascan (Applied Photophysics Limited, United Kingdom) three times at 80 nm/min with a 1 nm slit width and a time constant of 1 second. Data were collected from 190 nm to 350 nm at 1 nm intervals. Mannuronate and guluronate ratios were calculated using the equation described by Morris *et al*. (1980):

Mannuronate/guluronate ≈ 2.0 (peak/trough), if peak/trough < 1

% Mannuronate ≈ 27(peak/trough) + 40, if peak/trough > 1

3.6.9 Estimation of fucoidan average molecular weight and determination of sodium alginate molecular weight via intrinsic viscosity

Analytical ultra-centrifugation was performed to determine the molecular weights of the fucoidan. Fucoidan stock solutions (1 mg/ml) prepared in distilled water were transferred and filtered through dH_2O pre-washed 100 K, 50 K, 30 K, and 10 K Amicon® centrifugal filter units (Merck Millipore, Darmstadt, HE, Germany). The supernatants were obtained by centrifuging the filters at 13000 ×*g* for 10 minutes and were then analysed for the presence of fucoidan using the phenol-sulfuric acid assay. Filters were washed with 50 µl dH_2O and analysed for the presence of retained fucoidan in the same manner as the supernatants.

Sodium alginate stock solutions of 10 mg/ml were prepared in 100 mM saline water, and dilutions from 0.1 to 10 mg/ml were prepared from the stock solution. After vortexing, the solution for 5 seconds, 1 ml was added to 50- and 150-micro capillary diameter sized viscometers (Cannon Instrument Company, State College, PA, USA). The flow time of the solution from the top to the bottom meniscus was measured. The intrinsic viscosity was calculated by using the formulae: $\eta_r = \eta / \eta_r$, where η_r is the relative viscosity, η is the sodium alginate solution viscosity (mPa s), η_r is the solvent viscosity; $\eta_r = 1 + [\eta]C$, where $[\eta]$ is the intrinsic viscosity (L g^{-1}), η_r is the relative viscosity, C is the sodium alginate concentration. The following formula estimated the molecular weight of sodium alginate: $[\eta] = k[Mw]^a$, where k and a are sodium alginate-specific constants; $k = 7.3 \times 10^{-5}$ L/g and $a = 0.92$ (Halabalová et al., 2004).

3.6.10 Congo red polysaccharide folding analysis

A modified method of Congo red described by Yang *et al.* (2021) was performed. A 1 mL of 2 mg/mL sodium alginate solution was mixed with different concentrations of NaOH ranging from 0 – 0.5 mol/L (0, 0.025, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mol/l), then a Congo red stock solution (80 µmol/l) was added to the each NaOH solution. The mixture was incubated for 1 hour at 25°C. Water was used instead of sodium alginate solution for the control. The total absorbance was scanned from 450 to 510 nm using a PowerWaveX™ spectrophotometer with KC Junior software.

3.6.11 X-ray powder diffraction analysis

The crystallinity of the sodium alginate was measured using a Bruker D2 2nd Gen Phaser (Billerica, Massachusetts, USA). Samples were scanned from 2θ of 5 to 60° with a step size of 0.02° per second. The relative crystallinity of the polysaccharide was calculated by dividing the area of the diffraction peak at 2θ by the total area of 17 - 21°.

3.6.12 Recycling of spent ethanol

Ethanol that was used in the precipitation of fucoidan and sodium alginate was recycled by fractional distillation. A spent ethanol flask was connected to a fraction column, connected to a condenser with cold water, and then the condenser was connected to a collecting flask. The heating mantle was used to maintain the temperature of 200 ml of spent ethanol flask at 80˚C while stirring at 200 rpm; the amount of ethanol after the distillation was recorded.

3.7 Statistical analysis and workflow

3.7.1 Statistical analysis

Triplicate data sets were used in all experiments and expressed as means ± standard deviations (S.D.). Significant differences between extraction conditions and the yield of fucoidan and sodium alginate were determined by one-way analysis of variance (ANOVA). The ANOVA test was performed using the Data analysis feature in GraphPad Prism software version 6 (GraphPad Inc). The null hypothesis was considered invalid when the *P*-value was less than 0.05. A *t*-test was also done to compare the two groups and was deemed significant when the *P*-value was less than 0.05. Pearson's correlation was used to determine the linear relationship between pH and the yield of fucoidan and sodium alginate. Pearson's correlation was calculated according to Appendix B.6.

3.7.2 Summarised workflow of the optimisation of the extraction method

After each optimisation step, the optimal condition was selected and used throughout the study. The optimisation procedure applied is indicated as follows: pH of delipidation → temperature of delipidation → % ethanol for fucoidan precipitation → concentration of sodium carbonate for

sodium alginate extraction → % ethanol for sodium alginate precipitation. For example, pH x → x˚C → x% EtOH → x M Na_2CO_3 → x% EtOH, where x indicates the different variables investigated.

Chapter 4 – Results and Discussion

4.1 Optimisation of the co-extraction of fucoidan and sodium alginate

4.1.1 Effect of pH on the yield of fucoidan and sodium alginate

The impact of the pH of the delipidation solution on fucoidan and sodium alginate extraction was evaluated by preparing solutions with pH values ranging from 1 to 13, adjusted using 2 M HCl and 2 M KOH. Table 4.1 shows that acidic solutions significantly enhanced the extraction of fucoidan and sodium alginate compared to alkaline solutions. The data demonstrated a clear downward trend in yields for both compounds as the pH increased. Pearson's correlation coefficients of -0.759 for fucoidan and -0.899 for sodium alginate confirmed a strong negative correlation, indicating that yields decreased as the pH shifted from acidic to basic (Table 4.1).

The extraction of fucoidan showed notable differences between acidic and alkaline conditions. At pH 1.0, the fucoidan yield reached 3.51% (w/w), while at pH 13, the yield dropped to 1.42% (w/w) (Table 4.1). These values fall within the reported range of fucoidan yields, which are typically between 0.87% (w/w) and 9.46% (w/w) for extractions (Rani et al., 2017). Lorbeer et al. (2015) achieved a maximum fucoidan yield of 3.75% (w/w) in a sequential extraction study, reporting that a pH of 1.0 significantly improved fucoidan yields from *Ecklonia radiata*.

Table 4.1

Fucoidan and sodium alginate yields under different pH conditions during the delipidation step. Values are represented as means ± S.D. (n=3).

pH	Fucoidan		Sodium Alginate	
	(g)	(%)	(g)	(%)
1	0.263 ± 0.028	3.51	4.79 ± 0.4	63.91
2	0.244 ± 0.039	3.25	4.3 ± 0.29	57.11
3	0.219 ± 0.042	2.92	4.13 ± 0.32	55.02
4	0.187 ± 0.049	2.49	3.45 ± 0.52	46.0
5	0.177 ± 0.071	2.36	3.49 ± 0.57	46.52
6	0.16 ± 0.062	2.13	3.46 ± 0.67	46.09
7	0.147 ± 0.085	1.96	3.60 ± 0.621	48.04
8	0.189 ± 0.07	2.52	3.14 ± 1.154	41.81
9	0.123 ± 0.097	1.64	2.91 ± 1.1	38.78
10	0.159 ± 0.047	2.12	2.814 ± 0.547	37.52
11	0.103 ± 0.076	1.38	3.27 ± 1.06	43.59
12	0.175 ± 0.007	1.56	2.99 ± 1.09	39.80
13	0.16 ± 0.0566	1.42	1.77 ± 1.59	23.54

4.1.2 Effect of temperature on delipidation

The effect of temperature on fucoidan extraction during the delipidation step was assessed by varying the temperature between 10°C and 37°C (Table 4.2). The highest fucoidan yield of 3.73% (w/w) was achieved at 10°C, while the lowest yield of 3.28% (w/w) was observed at 37°C. Despite these differences, the yield variation across the temperature range was not statistically significant

($p > 0.05$). A *t*-test comparing yields at 22°C (typical room temperature) and 10°C also showed no significant difference ($p > 0.05$).

As a result, subsequent delipidation steps were conducted at room temperature for the remainder of the study. This finding is particularly advantageous since maintaining room temperature avoids the need for heating, thereby reducing energy costs without compromising the yield of fucoidan (Table 4.2).

Table 4.2

Effect of temperature on the yield of fucoidan during the delipidation step

Temperature (˚C)	**Fucoidan**	
	(g)	**(%)**
18	0.28 ± 0.013	3.73
22	0.257 ± 0.02	3.43
37	0.246 ± 0.015	3.28
50	0.26 ± 0.037	3.47

4.1.3 Effect of ethanol concentration on the precipitation of fucoidan

The impact of final ethanol concentration on fucoidan precipitation was evaluated using concentrations ranging from 20% to 90% (v/v) (Table 4.3). At a final ethanol concentration of 20%, no fucoidan precipitation was observed. Fucoidan yields of 0.098 g, 0.167 g, and 0.25 g were obtained at 50%, 70%, and 80% ethanol, respectively. At 90%, the yield increased marginally by 0.01 g, resulting in a total yield of 0.26 g, with no further increase observed above 90%. The difference in yield between 70% and 80% ethanol was significant at 32% (w/w), whereas increasing the ethanol concentration from 80% to 90% produced only an additional yield of 3.8% (w/w), which was considered negligible.

Based on these results, the optimal ethanol concentration for fucoidan precipitation was determined to be 80% (v/v), as it achieved 96.2% (w/w) of the maximum fucoidan yield, while minimising ethanol usage. These findings align with a previous study by Daub et al. (2020), who used an ethanol concentration of 85% (v/v) to achieve a fucoidan yield of 6.89% (w/w). While fucoidan yields can vary depending on factors such as seaweed species, seasonality, geographical location, maturity, and the part of the seaweed used, the yields obtained in this study are consistent with values reported by Lorbeer et al. (2015), Rani et al. (2017), and Filippo-Herrera et al. (2018).

The optimised method for extracting fucoidan involved using a pH 1.0 solution during the delipidation step and a final ethanol concentration of 80% (v/v) for precipitation, ensuring efficient extraction and alignment with previously reported methodologies.

Table 4.3

Effect of final ethanol concentration on the yield of fucoidan during the precipitation step

Ethanol concentration (%)	The yield of fucoidan at each ethanol concentration (g)	The cumulative yield of fucoidan (%)
20	0	0.0
50	0.098 ± 0.021	37.70
70	0.096 ± 0.032	64.20
80	0.083 ± 0.024	96.20
90	0.01 ± 0.001	100.0

4.1.4 Effect of Na_2CO_3 concentration

The effect of sodium carbonate concentration on sodium alginate yield was evaluated using concentrations ranging from 0 to 3 M (Table 4.4). The yields of crude sodium alginate and washed sodium alginate were determined, as washing was necessary to remove excess sodium carbonate bound to the alginate during extraction. Sodium alginate precipitation started to occur at 0.5 M

sodium carbonate, with yields of sodium alginate increasing at higher concentrations of sodium carbonate. At 2 M and 3 M sodium carbonate, the crude yields were 167% and 161% (w/w), respectively, indicating significant amounts of excess sodium carbonate bound to the precipitate.

After washing, the yields of sodium alginate decreased by 2.7%, 5.73%, 10.67%, 95%, and 86.33% (w/w), respectively, for sodium carbonate concentrations ranging from 0.5 M to 3 M. This showed that 0.5 M sodium carbonate was optimal, as it produced the smallest difference between washed and unwashed samples, minimising contamination and avoiding the need for excessive washing. In contrast, samples extracted with 2 M and 3 M sodium carbonate still produced yields of 72% and 74.67% (w/w) after washing, suggesting that excess sodium carbonate remained bound to the alginate.

The unwashed and washed sodium alginate samples precipitated with 0.5 M, 1.0 M, and 1.5 M sodium carbonate were analysed for their ash content using thermogravimetric analysis (TGA), with a PerkinElmer® Pyris Diamond Differential Scanning Calorimeter (DSC), over a temperature range of 30°C to 800°C under a nitrogen atmosphere (Fig. 4.1). Optimisation of sodium carbonate concentration was critical for the extraction process, as using too little sodium carbonate prevented complete conversion of alginate to sodium alginate, while excess sodium carbonate co-precipitated with the alginate, necessitating additional washing steps with ethanol and water.

The commercial sodium alginate, used as a control, decomposed in two distinct steps: the first at 100°C and the second at 300°C, leaving 22.4% ash content (Fig. 4.1A). The decomposition rate at 300°C appeared to determine the ash content, with purer sodium alginates exhibiting a steep increase in ash content at this temperature. Sodium carbonate, used in the extraction process, exhibited an ash content of 99%, as expected due to its high melting point of 851°C (Fig. 4.1B). This decomposition pattern was observed in all washed sodium alginate samples but not in the unwashed samples, except for the 0.5 M sodium carbonate concentration, where both washed and unwashed samples showed similar decomposition trends (Figs. 4.1C and D). Unwashed sodium alginate samples extracted with 1 M and 1.5 M sodium carbonate had high ash contents of 56.57% and 62.31%, respectively (Figs. 4.1E and G), indicating significant contamination with residual sodium carbonate. In contrast, their washed counterparts showed much lower ash contents of 22.86% and 24.19%, respectively (Figs. 4.1F and H). These results demonstrate the effectiveness

of the washing process in removing excess sodium carbonate and highlight the superior purity of the washed samples. These findings align with studies in the literature, where Viswanathan and Nallamuthu (2014) used 5% (w/v) sodium carbonate (approximately 0.47 M), and Lorbeer et al. (2015) used 0.2 M to achieve a 43.9% (w/w) yield.

Using 0.5 M sodium carbonate was deemed optimal in our study, yielding pure sodium alginate without the need for additional washing. At 3 M sodium carbonate, the yield of 161% (w/w) was unrealistic, indicating significant contamination from bound sodium carbonate. The average difference of 35.92% between washed and unwashed samples at this concentration highlighted the lack in efficiency when using higher amounts of sodium carbonate.

Table 4.4

Effect of sodium carbonate concentration on the yield of sodium alginate before and after washing

Na_2CO_3 concentration (M)	**Yield of sodium alginate**		**Yield of washed sodium alginate**		**Difference in sodium alginate yield (percentage difference, %)**
	(g)	**(%)**	**(g)**	**(%)**	
0	0	0	0	0	N/A
0.5	3.41 ± 0.325	45.50	3.21 ± 0.287	42.8	2.70
1.0	4.67 ± 0.468	62.26	4.24 ± 0.067	56.53	5.73
1.5	5.21 ± 0.609	69.47	4.41 ±0.133	58.8	10.67
2.0	12.59 ± 0.218	167.0	5.4 ± 0.424	72.0	95.0
3.0	12.11 ± 0.111	161.0	5.6 ± 0.549	74.67	86.33

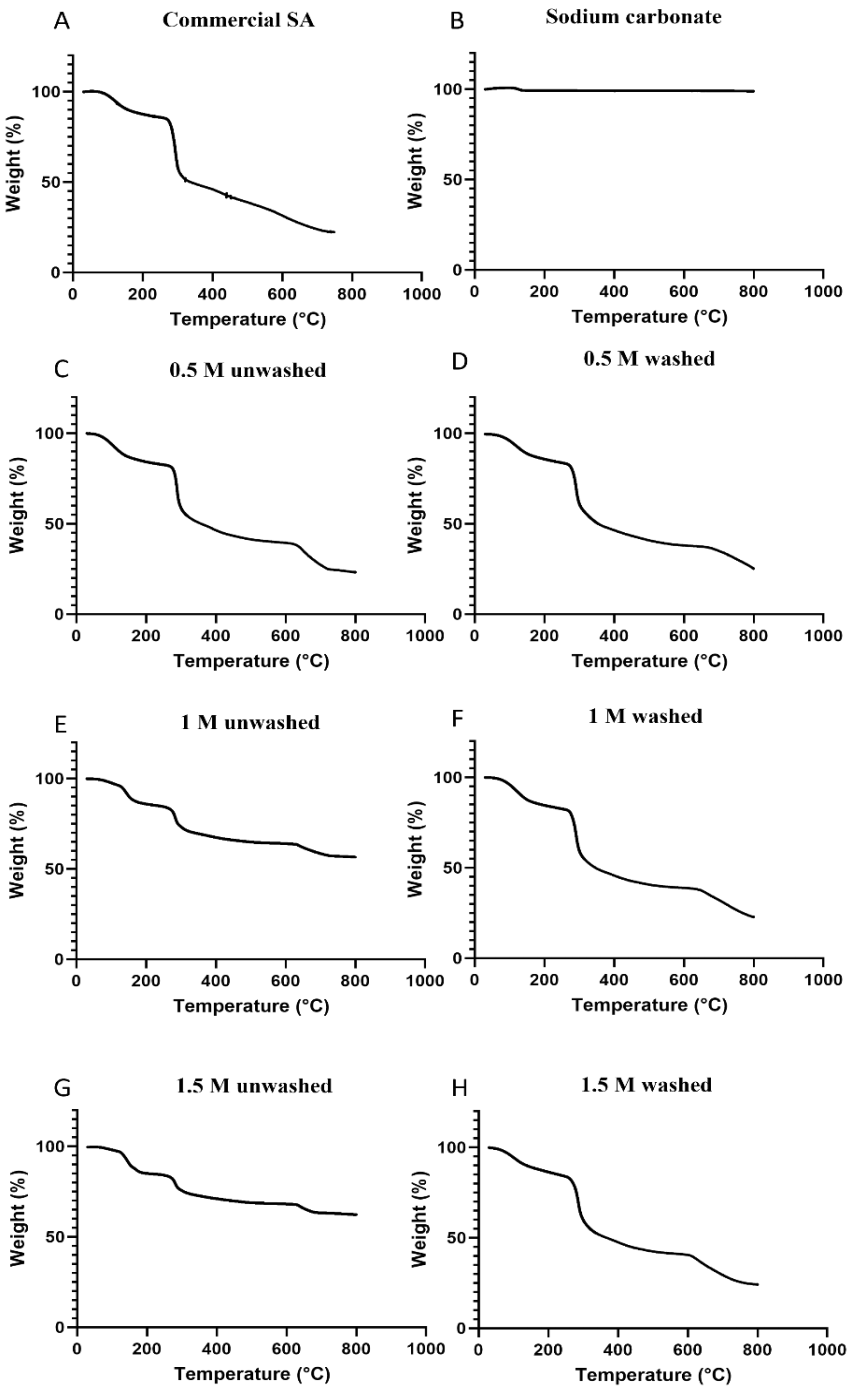


Figure 4.1: The thermogravimetric analysis of extracted sodium alginates under different concentrations of sodium carbonate. The commercial sodium alginate, sodium carbonate and the extracted sodium alginates at different concentrations were analysed on the thermogravimetric analyser (PerkinElmer®, Pyris Diamond DSC) from 30°C to 800°C under nitrogen. **A)** TGA profile of commercial sodium alginate. **B)** TGA profile of sodium carbonate. **C)** TGA profile of sodium alginate extracted with 0.5 M sodium carbonate, unwashed. **D)** TGA profile of sodium alginate extracted with 0.5 M sodium carbonate, washed. **E)** TGA profile of sodium alginate extracted with 1 M sodium carbonate, unwashed. **F)** TGA profile of sodium alginate extracted with 1 M sodium carbonate, washed. **G)** TGA profile of sodium alginate extracted with 1.5 M sodium carbonate, unwashed. **H)** TGA profile of sodium alginate extracted with 1.5 M sodium carbonate, washed.

4.1.5 Effect of ethanol concentration on the precipitation of sodium alginate

The optimal final ethanol concentration for sodium alginate precipitation was determined by evaluating concentrations ranging from 20% to 90% (Table 4.5). At 20% ethanol, 1.217 g of sodium alginate (24.8% w/w) precipitated. Additional yields of 2.203 g, 0.876 g, and 0.608 g were obtained at 50%, 60%, and 70% ethanol, respectively. No further sodium alginate precipitation was observed above 70% ethanol. The total yield of sodium alginate across all concentrations was 4.904 g.

The difference in yield between 60% and 70% ethanol concentrations was 12.4%, demonstrating that 70% ethanol was the optimal concentration for complete sodium alginate precipitation. This concentration effectively maximised yield while avoiding the unnecessary use of higher volumes of ethanol, ensuring efficiency in the extraction process.

Table 4.5

Effect of final ethanol concentration on the yields of sodium alginate during the precipitation step

Final ethanol concentration (%)	**Yield of sodium alginate (g)**	**Cumulative yield of sodium alginate (g)**	
		(g)	**(%)**
20	1.217± 0.098	1.217	24.80
50	2.203 ± 0.112	3.42	69.70
60	0.876 ± 0.189	4.296	87.60
70	0.608 ± 0.233	4.904	100.0
80	0	4.904	100.0
90	0	4.904	100.0

4.1.6 Yields of extracts from standard and optimised extraction methods

The optimised sequential extraction process for fucoidan and sodium alginate from *E. maxima* was established as follows: delipidation at pH 1.0 with 80% ethanol precipitation for fucoidan and extraction with 0.5 M Na_2CO_3, followed by 70% ethanol precipitation for sodium alginate (Fig. 4.2). Table 4.6 shows a slight improvement in fucoidan yield from 3.51% (w/w) to 3.67% (w/w) with this optimised process, while the sodium alginate yield decreased from 63.91% (w/w) to 58.7% (w/w), likely due to residual sodium carbonate.

Compared to previous studies, the optimised process produced yields that were similar or superior, particularly for sodium alginate, which outperformed all other reported yields by over 14% (w/w) (Table 4.6). However, the fucoidan yield from this study was lower than one of the results reported by Ptak et al. (2019), who extracted fucoidan from *Fucus vesiculosus*.

During the delipidation step, sodium alginate was extracted from the residual biomass (remaining after fucoidan extraction). The pH during delipidation was a key factor in determining fucoidan and sodium alginate yields. A pH of 1.0 produced the highest yields, with a sodium alginate yield of 63.82% (w/w), compared to only 23.54% (w/w) at pH 13, a decrease of nearly threefold. As the pH increased, the yield of sodium alginate declined progressively.

The yield of sodium alginate achieved in our study (at pH 1.0) exceeded the 40–47% (w/w) range reported in the literature (Rasmussen and Morrissey, 2007; Pal et al., 2014). This highlights the efficiency of the optimised method, which not only maximised yields but also demonstrated that our sequential extraction approach utilised the biomass more fully and effectively.

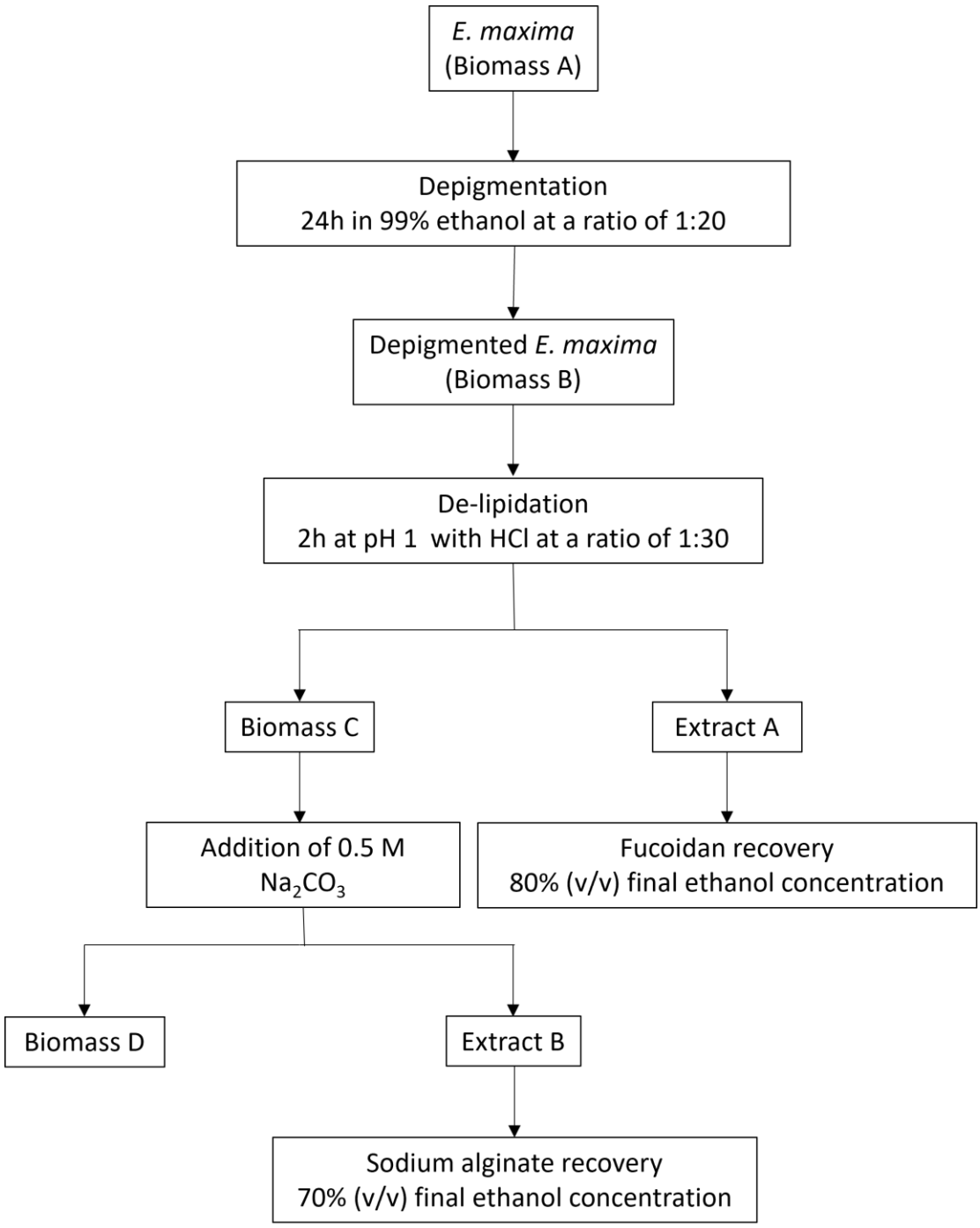


Figure 4.2: Scheme indicating the optimised sequential extraction of fucoidan and sodium alginate from the raw material to the final extracted products.

Table 4.6

Comparison of the fucoidan and sodium alginate extraction processes using the solvent method. Values are represented as means ± S.D. (n=3).

Type of seaweed	Fucoidan			Sodium Alginate			Reference
	pH	Final [EtOH] (%)	Yield (%)	[Na_2CO_3]	Final [EtOH] (%)	Yield (%)	
Ecklonia maxima	1.0 (HCl)	80	3.67	0.5 M	70	58.7	This study, optimised extraction method
Ecklonia radiata	1.0 (HCl)	67	3.75	0.2 M	50	44	Lorbeer *et al*., 2015
Fucus vesiculosus	1.87 (10 mM H_2SO_4)	70	11.1		N/A		Ptak *et al*., 2019
Padina tetrastromatica	1.52 (0.03 M HCl)	60	9.46		N/A		Rani *et al*., 2017
Sargassum cristaefolium	1-5 (HCl)	67	1.96	0.5 M	67	40.6	Sugiono and Ferdiansyah, 2020
Sargassum natans	2.0 (H_2SO_4)	N/A		0.5 M	94%	19	Mohammed *et al*., 2018

4.2 Large-scale extraction of fucoidan and sodium alginate

The total extracted masses of fucoidan and sodium alginate from *E. maxima*, along with their percentages relative to the starting biomass, are presented in Table 4.7. For fucoidan extractions (7.5–200 g starting mass), fucoidan yields ranged from 3.67% to 3.6% (w/w). The yield of fucoidan remained consistent regardless of the starting biomass, showing a linear relationship between extracted mass and starting biomass.

In contrast, the extraction yield of sodium alginate was much more variable. The highest sodium alginate yield of 61.09% (w/w) was obtained with a starting biomass of 100 g, while the lowest yield of 41.65% (w/w) was obtained with a starting biomass of 200 g, representing a difference of 19.44% (w/w). Sodium alginate yields generally decreased as the starting biomass increased, with small-scale (SS) extractions yielding 58.7% (w/w) compared to 41.65% (w/w) in large-scale (LS) extractions. This decline in sodium alginate yield at higher starting amounts of biomass is likely due to reduced stirring efficiencies, which may result in limited effective extraction and precipitation at larger biomass loads. While fucoidan extraction yields appeared to be unaffected by the amount of starting biomass, optimising the stirring and mixing efficiency at larger scales remains crucial for maintaining optimal yields of sodium alginate. These results highlight the challenges associated with scaling up sodium alginate extraction compared to fucoidan.

Table 4.7

Table showing the yields of fucoidan and sodium alginate from SS and LS extractions.
Values are represented as means ± S.D. (n=3).

Starting mass (Dry)	Fucoidan (%) (w/w)	Sodium alginate (%) (w/w)
7.5 (SS)	3.67	58.7
50	3.31	55.47
100	3.55	61.09
150	3.56	46.09
200 (LS)	3.6	41.65

4.3 Chemical composition analysis of fucoidan and sodium alginate

The chemical compositions of fucoidan and sodium alginate extracted from *Ecklonia maxima* from South Africa were analysed and compared to commercial standards of fucoidan (from *Fucus vesiculosus*) and sodium alginate. The extracts were derived from a starting mass of 7.5 g for SS and 200 g for LS, and the results are presented in Tables 4.8 and 4.9.

Fucoidan extracts were primarily composed of carbohydrates, with the SS extraction yielding a total sugar content of 69.45 ± 1.48% (w/w), while the LS extraction had a slightly lower total sugar content of 62.87 ± 0.98% (w/w). The predominant monosaccharide in *E. maxima* fucoidan was D-glucose, present at 9.18 ± 0.36% (w/w) in SS and 7.43 ± 0.58% (w/w) in LS. In comparison, the commercial fucoidan exhibited a lower total sugar content of 46.07 ± 1.83% (w/w), with L-fucose being the major monosaccharide at 7.92 ± 0.53% (w/w). Protein contamination was minimal in all samples, with LS fucoidan displaying the lowest protein content at 0.59 ± 0.08% (w/w). Phenolic content, which is often associated with sulphated polysaccharides, was highest in LS fucoidan (2.53 ± 0.79% (w/w)), followed by SS (1.94 ± 0.56% (w/w)) and the commercial standard (1.54 ± 0.89% (w/w)). The uronic acid content of fucoidan varied significantly, with the commercial fucoidan containing 16.27 ± 2.36% (w/w), while SS and LS fucoidan contained 29.96 ± 3.12% (w/w) and 25.38 ± 2.75% (w/w), respectively. These differences are likely due to species-specific variations and extraction conditions. Despite these differences, both SS and LS fucoidan displayed chemical compositions similar to those in reports in the literature. In addition, these samples were minimally contaminated by proteins and phenolic acids, with the exception of uronic acids.

Sodium alginate extracted from *E. maxima* was chemically comparable to the commercial standard. The total sugar content was 72.62 ± 5.3% (w/w) for the commercial sample, and 67.94 ± 1.2% (w/w) for SS, and 73.93 ± 2.3% (w/w) for LS. Monosaccharide profiles revealed minor differences, with LS sodium alginate containing slightly lower levels of D-fructose, L-fucose, and D-galactose compared to the commercial sample. Importantly, no protein was detected in the extracted sodium alginate samples, and phenolic content was low - with LS and SS extracts closely matching the commercial standard. The uronic acid content of sodium alginate showed no significant differences among the samples, with values of 119.89 ± 2.38% (w/w) for the commercial sample, 126 ± 6.8% (w/w) for SS, and 121.63 ± 3.9% (w/w) for LS. Although the

values of uronic acids of sodium alginates were higher than the theoretical maximum (100%), due to the sensitivity of the assay, the numerical values of the uronic acids were similar. These results confirm that the extracted sodium alginate is of high purity and chemically equivalent to commercial-grade sodium alginate.

The results highlighted the scalability of the extraction processes. While fucoidan extraction exhibited slight compositional differences between the SS and LS extraction processes (likely due to scaling factors), both methods produced high-purity extracts that were similar to or better than the commercial standards. Sodium alginate extractions, on the other hand, showed consistently similar chemical composition profiles and closely matched the chemical composition of the commercial standard. The findings of this study confirmed that the optimised extraction processes yielded fucoidan and sodium alginate of excellent quality, suitable for application in various industries, and comparable to existing commercial benchmarks.

Table 4.8
Chemical profiles of the fucoidans from this study and commercially available sample and their respective *P*-values

Fucoidan source	Total sugar (%)	Uronic acid (%)	Protein (%)	Sulphate (%)	Phenols (%)	Monosaccharide composition (%)						
						Fruc	**Fuc**	**Gal**	**GluA**	**Glc**	**Man**	**Xyl**
Commercial fucoidan from *F. vesiculosus*	46.07 ± 1.83	16.27 ± 2.36	1.47 ± 0.96	6.35 ± 0.63	1.54 ± 0.89	0.10 ± 0.04	7.92 ± 0.53	2.87 ± 0.23	1.06 ± 0.46	0.93 ± 0.27	0.27 ± 0.08	1.15 ± 0.06
Fucoidan *E. maxima* (SS)	69.45 ± 1.48	29.96 ± 3.12	1.1 ± 0.11	6.14 ± 0.44	1.94 ± 0.56	1.93 ± 0.47	4.26 ± 0.32	2.27 ± 0.86	0.87 ± 0.29	9.18 ± 0.36	0.42 ± 0.11	1.28 ± 0.44
Fucoidan *E. maxima* (LS)	62.87 ± 0.98	25.38 ± 2.75	0.59 ± 0.08	7.03 ± 0.47	2.53 ± 0.79	0.97 ± 0.08	6.1 ± 1.13	2.84 ± 0.39	1.1 ± 0.31	7.43 ± 0.58	0.33 ± 0.07	1.39 ± 0.42
P-value[1]	**0.0008**	**0.0001**	0.2254	**0.0180**	**0.0033**	**0.0007**	**0.0344**	0.7762	0.6900	**0.0008**	**0.0091**	0.3675
P-value[2]	**0.0019**	**0.0022**	**0.0012**	**0.0004**	**0.0471**	0.0509	0.0589	0.1705	**0.0025**	**0.0052**	0.0600	**0.0108**

* (SS), Small-scale 7.5 g; (LS), Large-scale 200 g; RS, reducing sugar; Fruc, D-fructose; Fuc, L-fucose; Gal, D-galactose; GluA, D-glucuronic acid; Glc, D-glucose; Man, D-mannose; Xyl, D-xylose

[1]-*P*-values of large-scale against the commercial standard and statistically significant values are highlighted in bold.

[2]-*P*-values of large-scale against the small-scale and statistically significant values are highlighted in bold.

Table 4.9
Chemical profiles of the sodium alginates from this study and commercially available sample and their respective *P*-values

Sodium alginate source	Total sugar (%)	Uronic acid (%)	Protein (%)	Sulphate (%)	Phenols (%)	Monosaccharide composition (%)						
						Fruc	Fuc	Gal	GluA	Glc	Man	Xyl
Commercial sodium alginate	72.62 ± 5.30	119.89 ± 2.38	0	0.05 ± 0.07	0.84 ± 0.64	1.1 ± 0.24	3.21 ± 0.67	1.1 ± 0.15	3.89 ± 0.99	1.68 ± 0.66	5.89 ± 1.1	0.66 ± 0.12
Sodium alginate *E. maxima* (SS)	67.94 ± 1.20	126 ± 6.80	0	0.84 ± 0.68	1.14 ± 0.66	0.89 ± 0.19	2.97 ± 0.20	0.67 ± 0.09	3.12 ± 0.85	1.06 ± 0.42	5.39 ± 0.87	0.31 ± 0.08
Sodium alginate *E. maxima* (LS)	73.93 ± 2.30	121.63 ± 3.90	0	0.12 ± 0.09	0.85 ± 0.23	0.83 ± 0.15	2.58 ± 0.71	0.89 ± 0.13	3.69 ± 1.1	1.89 ± 0.98	6.13 ± 1.21	0.27 ± 0.04
P-value[1]	0.5284	0.18590	N/A	**0.0261**	0.9701	**0.0351**	**0.0013**	**0.0030**	0.0878	0.3735	0.0634	**0.0137**
P-value[2]	**0.0111**	0.1208	N/A	0.1689	0.3632	0.1217	0.3164	**0.0108**	0.0585	0.1241	0.0637	0.2254

***** (SS), Small-scale 7.5 g; (LS), Large-scale 200 g; RS, reducing sugar; Fruc, D-fructose; Fuc, L-fucose; Gal, D-galactose; GluA, D-glucuronic acid; Glc, D-glucose; Man, D-mannose; Xyl, D-xylose

[1]-*P*-values of large-scale against the commercial counterpart and statistically significant values are highlighted in bold.

[2]-*P*-values of large-scale against the small-scale and statistically significant values are highlighted in bold.

4.4 Structural characterisation of fucoidan and sodium alginate

4.4.1 Functional group determination of fucoidan and sodium alginate by FTIR spectroscopy

The FTIR spectra of the fucoidans and sodium alginates extracted from *E. maxima* were analysed and compared to commercial standards. SS and LS extracts demonstrated strong similarities that confirmed their identity and quality. The fucoidan extracts, both at SS and LS, closely resembled commercial fucoidan, displaying all characteristic peaks expected of fucoidan (Fig. 4.3). A broad band at 3400 cm^{-1} indicated O-H stretching vibrations typical of polysaccharides (Wang and Chen, 2016). Peaks at 1600 cm^{-1} were attributed to asymmetric stretching vibrations of the carboxylate O-C-O bond, while the 1220 cm^{-1} region revealed the presence of sulphate ester groups, a characteristic peak of fucoidan. Intense peaks near 1000 cm^{-1} suggested guluronic and mannuronic acid residues, which may indicate residual alginate content, as described by Chale et al. (2014). In addition, the peaks in the 820 cm^{-1} region pointed to small amounts of sulphate groups in the C-2 and C-3 positions (Wang et al., 2010).

The spectra of *E. maxima* fucoidan extracts differed slightly from the commercial fucoidan from *F. vesiculosus*. Notably, the 1000 cm^{-1} peak, indicative of uronic acid residues, was more pronounced in *E. maxima* extracts, suggesting potential contamination from residual alginate. Despite this, the overall FTIR profiles of SS and LS fucoidan extracts were consistent with the commercial standard, confirming their quality and the presence of all key fucoidan-associated features.

Similarly, the FTIR spectra of the sodium alginate extracts from *E. maxima* were nearly identical to those of commercial sodium alginate (Fig. 4.4). The characteristic broad band at 3200 cm^{-1} indicated hydrogen-bonded O-H stretching vibrations of polysaccharides, while peaks at 1600 cm^{-1} and 1400 cm^{-1} were attributed to carboxylate O-C-O stretching and methylene C-H stretching, respectively. A distinct peak at 1025 cm^{-1} signified the presence of monosaccharide units, consistent with the sugar composition analysis (Table 4.9). Importantly, the absence of peaks in the 1230–1280 cm^{-1} range confirmed that the sodium alginate samples did not contain sulphated polysaccharides, excluding the possibility of fucoidan contamination (Rashedy et al., 2021).

A notable observation in the LS sodium alginate spectra was the pronounced peak at 1400 cm^{-1}, potentially indicating inefficient conversion of alginate to sodium alginate due to suboptimal mixing in the large-scale extraction process. This discrepancy was less pronounced in the SS samples, suggesting that scale-up inefficiencies may have contributed to this problem. Improved reactor design and enhanced mixing efficiency could address this problem and further optimise the process. Despite this minor difference, the overall profiles of SS and LS sodium alginate extracts were highly consistent with the commercial standard.

Although FTIR analysis effectively confirmed the identity of fucoidan and sodium alginate and their similarity to commercial standards, it could not provide detailed structural information about the mannuronic and guluronic acid residues. Subsequent NMR analysis was required to determine the presence and ratio of these uronic acids, ensuring a comprehensive characterisation of the alginate extracts. Overall, the FTIR spectra validated the extraction and high quality of fucoidan and sodium alginate from *E. maxima*.

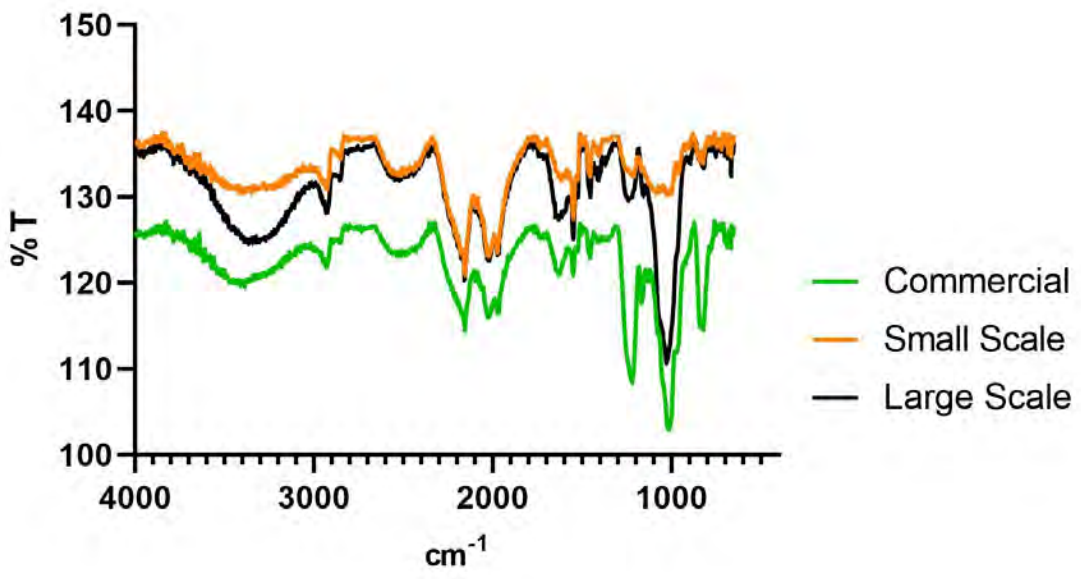


Figure 4.3: FTIR spectra of commercial, small- and large-scale extracted fucoidans

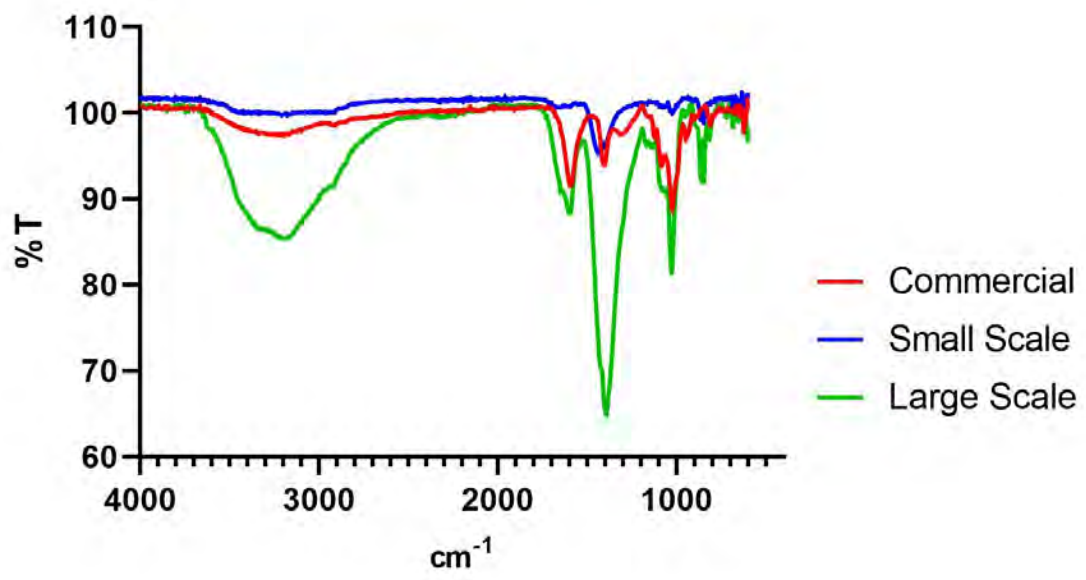


Figure 4.4: FTIR spectra of commercial, small- and large-scale sodium alginates

4.4.2 Determination of the M/G ratios in the sodium alginate extracts

The mannuronate and guluronate ratios in sodium alginate were confirmed using ^{1}H-NMR and circular dichroism (CD) spectroscopy. In the ^{1}H-NMR analysis, key peaks associated with guluronic acid and mannuronic acid residues were identified (Fig. 4.5). The anomeric proton of guluronic acid appeared at 5.1–5.2 ppm, the anomeric protons of mannuronic acid and H-5 of alternating blocks were observed at 4.7–4.9 ppm, and the H-5 of homopolymeric G blocks (guluronic acid residues) were present at 4.5–4.6 ppm. While all the key peaks were detected in the sodium alginate samples, the solvent peak of D_2O at approximately 4.75 ppm overlapped with signals from mannuronic acid and alternating blocks. However, the peaks at 4.5–4.6 ppm and 5.1–5.2 ppm clearly indicated the presence of homopolymeric guluronic blocks and guluronic acid units.

^{1}H-NMR spectroscopy confirmed the presence of guluronic acid units in both the SS and LS sodium alginate samples, as illustrated in Fig. 4.5. However, due to the prominent solvent peak at 4.75 ppm, the mannuronic acid residues could not be accurately quantified using NMR alone. Consequently, the M/G ratios were determined using CD spectroscopy, which provided additional

insight into the sodium alginate composition. This combined analytical approach ensured a comprehensive characterisation of the sodium alginate extracts, demonstrating their structural integrity and similarity to known standards.

Figure 4.6 illustrates the CD spectra for SS and LS, and commercial sodium alginate samples, all of which exhibited positive and negative Cotton effects at 199 nm and 213 nm. These features are indicative of helix-like and sheet-like structures, respectively, as described by Sun et al. (2018). Notably, while the SS and LS sodium alginate samples showed consistent peaks at 199 nm and troughs at 213 nm, the commercial sodium alginate displayed distinct values in these regions, reflecting differences in structural conformations. The structural variations likely stem from differences in the M/G ratios, which are influenced by the source organism, extraction methods, and environmental factors such as harvest season (Maurstad et al., 2007).

The mannuronate-to-guluronate (M/G) ratios of sodium alginates were determined using the method described by Morris et al. (1980), revealing significant differences between commercial sodium alginate and extracts from *Ecklonia maxima*. The commercial sodium alginate had an M/G ratio of 0.76, corresponding to 43% mannuronate and 57% guluronate, with a peak at -5.63 and a trough at -9.08. In contrast, the SS sodium alginate exhibited an M/G ratio of 1.89, equating to 65% mannuronate and 35% guluronic acid, while the LS sodium alginate closely matched SS sodium alginate, with an M/G ratio of 1.91 (66% mannuronate and 34% guluronate), showing a peak at -0.4 and a trough at -8.14. These results confirmed that the structural conformation and M/G ratios of sodium alginate extracted from *E. maxima* remained consistent during scale-up, demonstrating the robustness of the extraction method. Table 4.10 highlights some M/G results reported using ^{1}H-NMR or HPLC. The M/G ratio of the commercial sodium alginate in this study deviated from the values reported in some studies, such as the 3.42 ratio reported by Belattmania et al. (2020). However, the *E. maxima* sodium alginate ratios were comparable to those of *Laminaria digitata*, a brown seaweed species with an M/G ratio of 1.12 (Fertah et al., 2014). The M/G ratio of *E. maxima* from Darko et al. (2024) was reported to be 0.24, which was low compared to the ratio obtained for our study, as well as the M/G ratio of 1.18-1.59 obtained for *E. maxima* by Lorbeer *et al*. (2016). These findings highlight the reproducibility and consistency of the extraction process for *E. maxima*, ensuring the stable retention of mannuronic and guluronic block sequences in our study.

The higher mannuronic acid content in *E. maxima* sodium alginates compared to the commercial standard highlighted species-specific variations. According to Saji et al. (2022), M/G ratios reported for different seaweed species range from 0.43 to 2.52, with varying impacts on the physical properties of sodium alginate. Therefore, the commercial sodium alginate, with its lower mannuronic acid content, likely originates from a species with different biochemical characteristics, while the *E. maxima* sodium alginates, with a higher mannuronic acid content, exhibit distinct structural and functional properties.

Overall, the CD spectroscopy analysis confirmed that the M/G ratios and structural conformations of sodium alginate from *E. maxima* were unaffected by the scale-up process, which, in turn, ensured consistent quality and performance of the extracted sodium alginates. This method provided a deeper understanding of the composition and conformation of the polysaccharides and highlighted the effectiveness of the optimised extraction protocol.

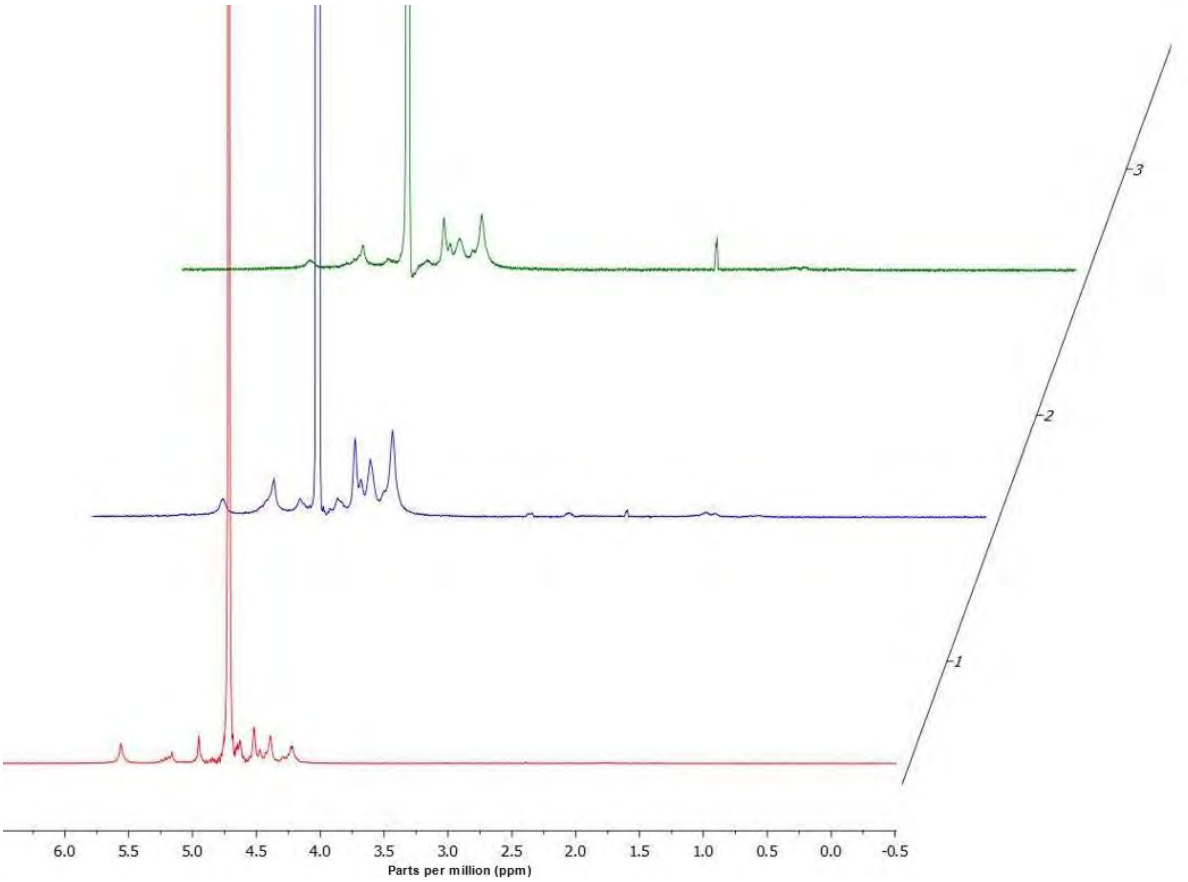


Figure 4.5. Nuclear magnetic resonance spectra of sodium alginate samples. Red represents the commercial sodium alginate, blue the small-scale, and green the large-scale sodium alginate from *E. maxima* using the optimised extraction method.

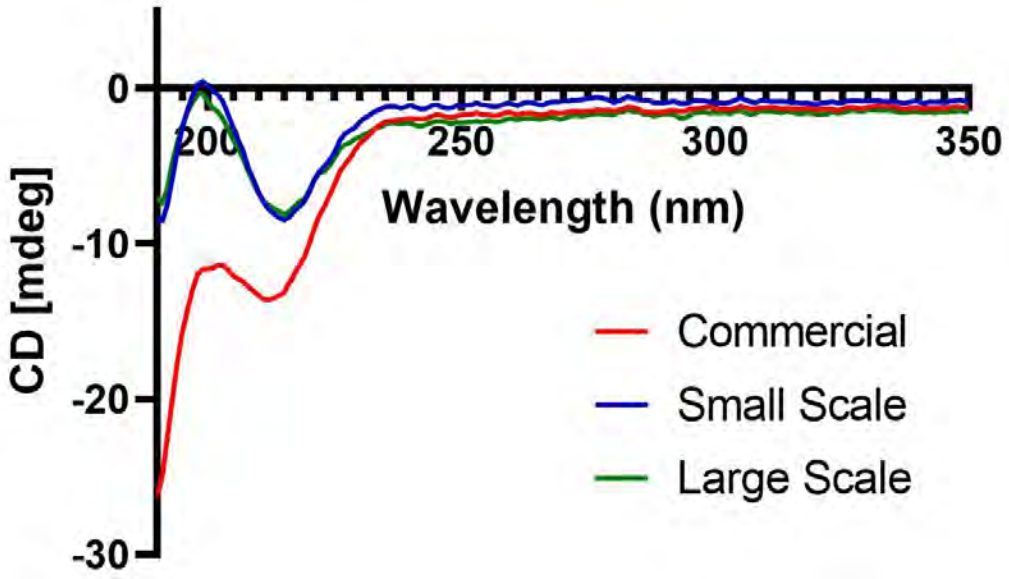


Figure 4.6: Circular dichroism absorbance of commercial sodium alginate, small-scale sodium alginate, and large-scale sodium alginate

Table 4.10
Mannuronic and guluronic compositions of sodium alginate extracted from *E. maxima* and commercial sodium alginate compared to other brown seaweeds via NMR spectroscopy or HPLC

Species	F_G	F_M	*M/G*	F_{MM}	F_{GG}	$F_{GM/MG}$	**Reference**
Ecklonia maxima	0.35	0.65	1.86	-	-	-	This study
Laminaria digitata	0.47	0.53	1.12	0.47	0.41	0.06	Fertah *et al*. (2014)
Sargassum aquifolium	0.07	0.93	12.96	0.87	0.01	0.06	Rashedy *et al*. (2021)
Ecklonia maxima	0.81	0.19	0.24	0.08	0.69	0.07	Darko *et al*. (2024)
Ecklonia radiata	-	-	1.18-1.59	-	-	-	Lorbeer *et al*. (2016)
Sargassum fluitans	0.85	0.15	0.20	0.11	0.80	0.04	Darko *et al*. (2024)
Sigma-Aldrich Na-Alginate	0.23	0.77	3.42	0.68	0.14	0.09	Belattmania *et al*. (2020)
Commercial Na-Alginate	0.57	0.43	0.76	-	-	-	This study

Where F_G, Guluronic acid fraction F_M, Mannuronic acid fraction *M/G*, Mannuronic/guluronic acid ratio; F_{MM}, Mannuronic homopolymeric blocks; F_{GG}, Guluronic homopolymeric blocks; $F_{GM/MG}$, heteropolymeric blocks of mannuronic and guluronic.

4.4.3 Molecular weight determination of fucoidan and sodium alginate

The molecular weight of fucoidan is highly polydisperse, meaning it encompasses a wide range of molecular weights from the same source rather than a singular value (Fitton et al., 2015). Fucoidans can be categorised into low-molecular-weight (<10 kDa), medium-molecular-weight (10–10,000 kDa), and high-molecular-weight (>10,000 kDa) types (van Weelden et al., 2019; Zayed et al., 2020). In this study, fucoidan extracted from *Fucus vesiculosus* had a molecular weight of Mw >100 kDa, which is in agreement with previously reported values, while fucoidan from *E. maxima* exhibited a molecular weight in the range of 10–50 kDa. The molecular weight of *F. vesiculosus* fucoidan of 105.4 kDa agreed with the findings of Sichert et al. (2020). However, the molecular weight of *E. maxima* fucoidan differed from the values reported by Sichert et al. (2020) and Lorbeer et al. (2015), who reported molecular weights of 123.5 kDa and 115 kDa, respectively. Instead, the molecular weight of *E. maxima* fucoidan in this study was comparable to that of *Laminaria japonica*, which showed a molecular weight of 10.5 kDa, coupled with a high fucose and sulphate content (Zayed et al., 2020).

The molecular weight of sodium alginate was determined using the viscometry and Mark-Houwink-Sakurada equation, where the constant *k* was adjusted based on the M/G ratios calculated earlier. The commercial sodium alginate had a molecular weight of 326 kDa, while the SS sodium alginate had a molecular weight of 429 kDa, and the LS sodium alginate displayed the highest molecular weight at 447 kDa. This variation highlights the impact of extraction scale and conditions on molecular weight. The viscosity data, which varied significantly across samples, influenced the molecular weight calculations, consistent with the method described by Vold et al. (2006).

The molecular weights of sodium alginate extracts were higher than those reported for *Laminaria digitata* by Fertah et al. (2014) and Vauchel et al. (2008), where the molecular weights were 114 kDa and 105 kDa, respectively. However, these were comparable to the higher molecular weights found in *Ecklonia radiata* (373–986 kDa) and *Sargassum cristaefolium* (194.1 kDa), as reported by Lorbeer et al. (2015) and Sugiono and Ferdiansyah (2020). These results suggest that the molecular weight of sodium alginate varies considerably among brown seaweed species and is influenced by factors such as the extraction process, environmental conditions, and the

biochemical composition of the source material. In addition, based on the results obtained by Darko *et al*. (2024) for the molecular weights from *Ecklonia maxima* and *Sargassum fluitans*, it appears as if sodium alginates extracted at ambient temperature have higher molecular weights than when extracted with heat and acid treatment. Sodium alginate from *E. maxima* at ambient temperature had a molecular weight of 1506 kDa, while that extracted using heat and 0.05 M HCl, had a molecular weight of 56 kDa (Darko *et al*., 2024).

The molecular weights of the fucoidan and sodium alginate extracted from *E. maxima* fell within the ranges reported previously for certain seaweed species, highlighting the inherent variability in polysaccharides across various sources. These findings highlighted the importance of extraction methods and source species in determining the molecular weight and associated functional properties of these biopolymers.

4.4.4 Congo red and X-ray powder diffraction

Congo red spectroscopy and X-ray powder diffraction (XRD) were employed to analyse the structural morphologies of commercial and extracted sodium alginates, specifically focusing on their triple-helix conformation (Fig. 4.7 and Fig. 4.8). Congo red, known for forming complexes with helical polysaccharides, was used to confirm the triple-helix structure, while XRD provided insights into the ordered molecular arrangement of the polysaccharides.

Congo red spectroscopy results revealed a bathochromic shift for all sodium alginate samples, with maximum absorption wavelengths between 495 nm and 499 nm. This shift, compared to the spectrum of pure Congo red (which does not display a bathochromic shift in the 400–600 nm range), confirmed that all sodium alginate samples, including the commercial, SS and LS extracts, adopted a triple-helical conformation (Guo et al., 2018). The observed shift is in agreement with previous studies, which indicated that polysaccharides exhibit a triple-helical structure and interact with Congo red to produce this characteristic spectral change (Yang et al., 2021).

X-ray powder diffraction was used to compare the crystallinity of sodium alginate extracts to that of commercial sodium alginate. The diffraction patterns of SS and LS sodium alginates were found

to be very similar to each other, as shown in Fig. 4.8. These patterns also displayed characteristic ‘bun-shaped’ diffraction peaks in the 2θ range of 17–20°, indicating that both the SS and LS sodium alginates exhibited an amorphous structure. This observation was consistent with previous studies reporting the amorphous nature of sodium alginate in this 2θ range (Wang et al., 2014; Helmiyati and Aprilliza, 2017).

The commercial sodium alginate also demonstrated a ‘bun-shaped’ diffraction peak in the same 2θ range, confirming its amorphous structure. However, subtle differences were observed between the diffraction patterns of the commercial sodium alginate and the sodium alginate extracts from *E. maxima*. These differences suggest that the molecular arrangement of sodium alginate can vary based on which seaweed species is used as the source material. While the SS and LS sodium alginate extracts exhibited nearly identical diffraction patterns, indicating consistency in structural properties during scale-up, the commercial sodium alginate's pattern differed slightly, reflecting species-specific variations in X-ray diffraction patterns. These findings emphasise that sodium alginate's crystallinity and structural characteristics are influenced by the origin of the seaweed, the extraction process, and the molecular composition of the polysaccharide. The XRD results confirm the amorphous nature of sodium alginate from *E. maxima* and its structural similarity between various scales of extraction, further validating the quality and reproducibility of our extraction method.

XRD analysis further supported the Congo red findings by examining the molecular structure and arrangement of the sodium alginates. This technique, which detects ordered repeating units within polysaccharides, revealed patterns consistent with the presence of a triple-helical structure. The diffraction patterns obtained highlighted the ordered structural morphology of the sodium alginates, demonstrating their ability to maintain conformational stability across various scales of extraction.

Together, the Congo red and XRD results confirmed that all sodium alginate samples from *E. maxima*—whether extracted on a small or large scale—possessed a stable triple-helical structure similar to commercial sodium alginate. These findings validate the structural integrity and quality of the extracted sodium alginates, emphasising their potential for applications requiring polysaccharides with helical conformations.

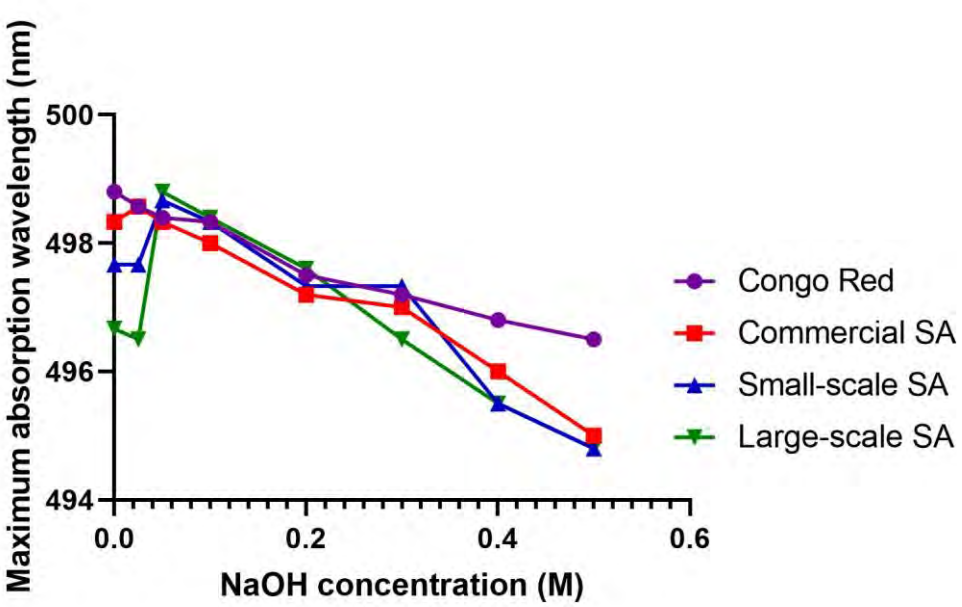


Figure 4.7: Spectroscopy of Congo red; commercial sodium alginate, small-scale sodium alginate, and large-scale sodium alginate. SA = sodium alginate.

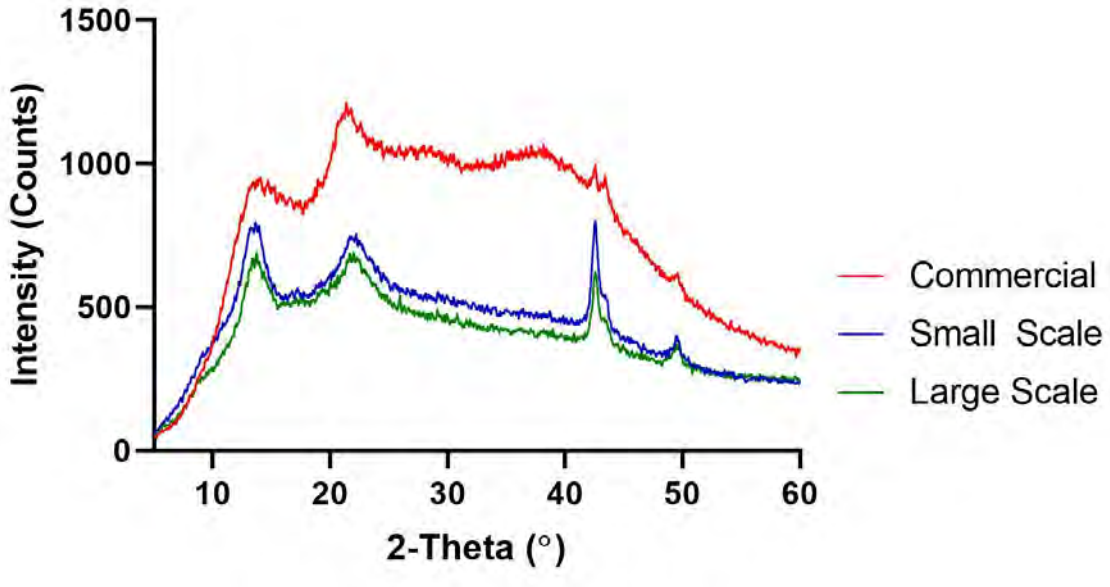


Figure 4.8: X-ray diffraction patterns of commercial sodium alginate, small-scale sodium alginate, and large-scale sodium alginate.

4.4.5 Distillation of spent ethanol

One of the most resource-intensive components of the process was the ethanol requirement for fucoidan and sodium alginate precipitation, which consumed approximately 6 litres and 8 litres of 99% ethanol per 100 g of dry seaweed during each respective step, excluding the depigmentation stage. To create a more sustainable extraction method for fucoidan and sodium alginate, the spent ethanol from the process was distilled and reused. After completing the precipitation steps, the liquid waste was pooled, and 200 ml was distilled. This process yielded 113 ml of ethanol, equivalent to 56.5% of the initial ethanol volume (equivalent to 7,910 ml per 100 g of dry seaweed). The reclaimed ethanol was sufficiently pure to be reused in subsequent extraction processes, demonstrating a practical step towards reducing chemical waste.

The ability to reclaim and reuse such a significant proportion of ethanol not only reduces the environmental impact of the process but also improves its cost efficiency. Further optimisation of ethanol recovery could enhance sustainability and affordability, making this greener approach a viable option for the large-scale commercial extraction of fucoidan and sodium alginate.

4.5 Conclusions

In this chapter, we have demonstrated an optimised method for the sequential extraction of fucoidan and sodium alginate from *Ecklonia maxima*, ensuring high yields, reproducibility, and scalability. By systematically investigating the effects of key parameters such as pH, temperature, ethanol concentration, and sodium carbonate concentration, the study identified conditions that maximise extraction efficiency while maintaining the structural integrity and purity of the bioactive compounds.

The results revealed that acidic conditions (pH 1.0) during the delipidation step and an ethanol concentration of 80% for fucoidan and 70% for sodium alginate precipitation were optimal, achieving yields of 3.67% (w/w) and 58.7% (w/w), respectively. The sodium carbonate concentration of 0.5 M proved ideal for sodium alginate extraction, minimising contamination and reducing the need for extensive washing.

Key findings from this chapter also highlighted significant variations in the molecular weights, M/G ratios, and chemical compositions of the extracted compounds. Fucoidan from *E. maxima* exhibited molecular weights ranging between 10–50 kDa, differing from commercial standards and previously reported values, while sodium alginate demonstrated a higher mannuronic acid content, and an M/G ratio of 1.86, indicative of its unique functional properties. The scalability of the extraction method was validated, with LS extractions producing consistent results comparable to SS extractions, emphasising the robustness of the developed protocol.

FTIR, ^{1}H-NMR, and CD spectroscopy analyses confirmed the chemical and structural integrity of the extracted compounds, which closely resembled commercial standards while showing species-specific variations. XRD and Congo red analysis further validated the structural stability of sodium alginate, confirming its triple-helical conformation and amorphous morphology.

In addition, the integration of ethanol distillation and recovery significantly reduced chemical waste, enhancing the sustainability and cost-effectiveness of the extraction process. These findings position the optimised protocol as a viable and eco-friendly approach for the commercial production of high-purity fucoidan and sodium alginate, meeting the growing demand across diverse industries, including pharmaceuticals, cosmetics, and nutraceuticals.

In conclusion, this chapter has established a comprehensive framework for the efficient extraction of bioactive compounds from *E. maxima*, paving the way for future research into their functional applications and expanding their potential contributions to the seaweed bioeconomy.

Chapter 5 – General Discussion, Conclusions and Future Perspectives

The aim of this study was to address the knowledge gap in scaling up the co-extraction of fucoidan and sodium alginate from the endemic South African brown seaweed *Ecklonia maxima,* to characterise the products and compare them against commercially available standards. An attempt to made to establish the relationship between chemical composition and structure of the extracted fucoidans and sodium alginates.

Fucoidan and sodium alginate were successfully co-extracted from the brown seaweed, *Ecklonia maxima*, found on western and southern coasts of South Africa. As fucoidan is known in the literature to be an important compound with many bioactivities (Daub *et al.,* 2020; Mabate *et al.,* 2021); and because sodium alginate has been widely used as a gelling polymer, and in recent years also as a bioplastic, it may be commercially viable to consider the co-extraction of these compounds. The extraction process used in this study underwent optimisation at each step with the ultimate goal of obtaining the highest yields of the products.

The optimal condition for the co-extraction of fucoidan and sodium alginate was delipidation under acidic conditions (pH 1.0), at room temperature, 80% (v/v) final ethanol concentration to precipitate fucoidan, 0.5 M sodium carbonate addition for alginate to sodium alginate conversion, and 70% (v/v) final ethanol concentration to precipitate the sodium alginate. These optimal conditions yielded 3.67% (dry w/w) fucoidan and 58.7% (dry w/w) sodium alginate as products. This resulted in the highest sodium alginate yield compared to the yields reported in the literature for sodium alginate to date. Lorbeer *et al*. (2015), when co-extracting fucoidan and sodium alginate, reported yields of 3.75% (dry w/w) and 44% (dry w/w), respectively, while Rani et al. (2017) reported a 9.46% (dry w/w) yield for fucoidan extraction at a final concentration of ethanol of 60% (v/v) during precipitation.

It is important to mention that different genera and species of seaweed exhibit different fucoidan and sodium alginate yields during the harvest season, depending on which part of the seaweed is used for extraction (Fletcher *et al*., 2017; Rani *et al*., 2017). To obtain more accurate values for yields of the co-extracted fucoidan and sodium alginate, the use of a larger industrial stainless-steel vessel with a strong impeller and a jacketed temperature control system is recommended; this

will also translate directly into a feasible commercial extraction of compounds from *Ecklonia maxima.*

The chemical composition results for the samples obtained from the large-scale (LS) extract of fucoidan and sodium alginate from *Ecklonia maxima* were very similar to that of the small-scale (SS) extract of fucoidan and sodium alginate, and the commercial (*F. vesiculosus*) standard. Fucoidan was extracted with a few undesirable contaminants, such as proteins and phenolics. The fucoidan from *Ecklonia maxima* and the commercial fucoidan consisted largely of D-glucose and L-fucose. Sodium alginate was extracted with almost no contaminants, such as proteins, sulphate, and phenolic compounds, indicating that the optimised co-extraction process was indeed robust and efficient. Sodium alginate consisted mainly of uronic acids such as mannuronic acid and guluronic acid, which were detected by the carbazole assay. However, because the carbazole assay determined the uronic acid contents to be greater than the theoretical maximum (100%), quantification by HPLC analysis is recommended for future studies as these will quantify the uronic acids more accurately.

FTIR of LS fucoidan and sodium alginate extracts showed similar profiles to those of the SS and commercial samples. All the expected peaks for polysaccharide, carboxylate, and sulphate ester groups, 3400 cm^{-1}, 1600 cm^{-1}, and 1220 cm^{-1}, respectively, were present in the fucoidan extracts. However, peaks around 1000 $cm^{-1,}$ indicative of guluronic and mannuronic acid residues resulting from contamination with sodium alginate, were observed. These may be removed by further optimising the fucoidan extraction process or by performing the delipidation step for longer. FTIR analysis of the LS sodium alginate sample also revealed all the expected peaks usually observed in a typical sodium alginate sample, i.e. carboxylate groups, monosaccharide units, and uronic acids. The sulphate ester group peak, a typical characteristic of fucoidans, was absent, confirming the extraction of pure sodium alginate, again providing evidence that the optimised co-extraction process was indeed robust.

The ratio of the relative abundance of mannuronic and guluronic acid residues of sodium alginate was further determined through ^{1}H-NMR. However, deuterium oxide (D_2O) caused a peak at 4.75 ppm during NMR analysis, which hindered the M/G ratio calculation. The solvent peak problem persisted even after several troubleshooting steps, such as pre-heating the sample and attempting to dissolve the sodium alginate with other solvents. Although NMR is one of the most accurate

and rapid ways of determining sodium alginate's M/G ratios, we determined the M/G ratio via circular dichroism (CD) spectroscopy because performing CD spectroscopy on sodium alginate will produce positive and negative Cotton effects at 199 nm and 213 nm, the peak and the trough can be used to determine the M/G ratio through an equation (Morris *et al*., 1980; Sun *et al*., 2018). Using CD spectroscopy, the M/G ratios of commercial sodium alginates, SS, and LS were 0.76, 1.89, and 1.91, respectively. As indicated in section 4.4.2, the M/G ratio for the commercial sodium alginate in this study was different from that reported by Belattmania *et al.* (2020). Therefore, more accurate data for the sodium alginates from *E. maxima* needs to be obtained in ^{1}H-NMR with a better experimental design. However, the SS and LS sodium alginates showed similar results during CD spectroscopy analysis, which indicated that both positive and negative Cotton effects, which can be used to determine the conformation and allow the calculation of the M/G ratio, were apparent in the alginate solution and as the species of seaweed for the commercial sodium alginate is not known, it can be assumed that the M/G ratios differed (0.76 for the commercial and 1.91 for the LS sodium alginate), as a result of them being sourced from different species.

The molecular weight of fucoidan was determined via molecular-weight-cut-off filters, and that of sodium alginate was determined via viscometry. Therefore, obtaining the exact molecular weights of fucoidans was challenging and was broadly categorised. However, the estimated molecular weight of the commercial fucoidan was in agreement with the reported molecular weight for this standard in the literature, but the molecular weights for the fucoidans of *E. maxima,* approximately 10-50 kDa, were not in agreement with the reported molecular weights and were shown to be lower than those reported in the literature – i.e. 123.5 and 115 kDa for *E. maxima* (Lorbeer *et al.* 2015; Sichert *et al.* 2020).

The molecular weights of sodium alginate were determined via intrinsic viscosity by using a viscometer. The Mark-Houwink-Sakurada equation was used to determine the molecular weight of sodium alginate using its intrinsic viscosity values. The result obtained via intrinsic viscosity, 447 kDa, was comparable to those reported in the literature, 105-114 kDa, for *Laminaria digitata*; however, due to the high viscosity characteristics of sodium alginate, other studies found it difficult to accurately measure the viscosity, and the errors were too high at times using different concentrations (Vauchel *et al*., 2008; Fertah *et al*., 2014). Because of the nature of these compounds, being polydisperse and high viscosity, more accurate molecular weight investigations are required.

Methods such as liquid chromatography-mass spectrometry (LC-MS), high-performance size exclusion chromatography with a refractive index detector and an evaporative light scattering detector (HPSEC-ELSD), or high-performance size exclusion chromatography with multi-angle laser light scattering (HPSEC-MALLS) may be used to investigate the molecular weights of these compounds further.

The quaternary structure and morphology of sodium alginate were further investigated via Congo red and X-ray powder diffraction, respectively. Congo red confirmed that the sodium alginate extracts were in a triple-helical structure form. XRD has also been used to confirm the amorphous structural character of sodium alginate extracts compared to commercial sodium alginate by demonstrating the 'bun-shaped' XRD curves.

The requirement for significant amounts of energy and solvent throughout the extraction process was addressed to ensure a greener extraction method from seaweeds. In particular, large amounts of ethanol were used to precipitate fucoidan and sodium alginate. The use of ethanol will increase proportionally as the starting mass of seaweed increases. Ethanol used in the extraction process was collected and fractionally distilled in the laboratory, recovering a little more than half of the ethanol, i.e. 56.5%.

This study focused on optimising the extraction process, troubleshooting, scaling up, and comparing the extracts against commercial standards. There is a growing demand for sulphated polysaccharides and alginates from brown algae and for the conservation of their structure to retain their biological properties (Hifney *et al.,* 2015).

Fucoidan has been used widely in pharmaceutical and nutraceutical applications such as in nanomedicine, and as anti-cancer, anti-coagulant, anti-viral and anti-inflammatory agents (Chollet *et al.,* 2016; Van Weelden *et al.,* 2019; Zayed and Ulber, 2020). Although no FDA-approved fucoidan-based drugs have reached the market for end-users as of 2022, except for supplements, there is a high demand in the pharmaceutical industry for the biological properties of fucoidan (Wijesinghe and Jeon, 2011). For example, heparin, a highly sulphated polysaccharide, is a well-known drug for its anti-coagulant activity; however, there have been cases of excessive bleeding and heparin-induced thrombocytopenia (Warkentin and Greinacher, 2013). Therefore, fucoidan, another highly sulphated polysaccharide, has been in demand as an alternative to heparin (Wijesinghe and Jeon, 2011). In 2016, Marinova Pty. Ltd had gained FDA approval to use *Fucus*

vesiculosus fucoidan as food additives in foods such as baked goods, soups, snack foods, imitation dairy products and seasonings and flavours for use at levels up to 30 mg/serving (FDA GRAS Notice 661, 2016). Maritech®, a subsidiary of Marinova Pty. Ltd has been producing and selling fucoidan products in dietary supplements, cosmetics, skincare and animal feeds (Maritech Fucoidan Product Portfolio, 2022). There is a significant interest among producers and the public in the health-promoting properties of fucoidan-containing foods. Over the years, much research has been performed on the biological properties of fucoidan and its health benefits, which will only ensure that fucoidans will be used more in industry (Wijesinghe and Jeon, 2011).

According to UN Comtrade, the seaweed-based hydrocolloids export market in 2019 reached US$ 1.74 billion (UN Comtrade, 2019). It is estimated that 30,000 tons of alginate are produced around the globe annually, with six major producing countries China, the USA, the UK, Japan, Chile and Germany, having a predicted market value of US$1.07 billion by 2028 with a compound annual growth of 5% (Saji *et al.,* 2022). These sodium alginates are used in the pharmaceutical and biomedical industries and in cosmetics, food, fertilisers, and water treatment (Lee and Mooney, 2012; Wang *et al.,* 2013; Pereira and Cotas, 2020; Shen *et al.,* 2020). In the biomedical field, alginate is widely used for its skin wound-healing properties due to bioactive compounds such as amino acids, alkaloids, tannins, flavonoids and phenols, which assist with wound healing (Janarthanan and Senthil Kumar, 2019). Alginate has been used in fibre, hydrofibre, hydrogels, films and foams in commercially available wound dressings (Łabowska *et al.,* 2019). Sodium alginate is also used to deliver a pill in a controlled manner in the treatment of diabetes mellitus, liver and parathyroid disease, and repair and regeneration of the liver (Lee and Mooney, 2012; Łabowska *et al.,* 2019). Furthermore, sodium alginates are used in cosmetics for their gel formation and thickening properties. Therefore, it is used in lipsticks to retain the colour of the lips and in lotions to retain water content in the body (Giridhar Reddy, 2022). Alginates are also employed to make anti-ageing masks as potential therapeutic fillers (Mori *et al.,* 2020; Giridhar Reddy, 2022).

The brown seaweed *Ecklonia maxima* is abundant in South Africa and is under-exploited for its bioactive compounds globally (Ferdouse *et al.*, 2018). A recent study by Zhang and Zhao (2020) revealed an increase in the number of studies conducted on alginate and other polysaccharides over the past decade due to a growing awareness of using sustainable materials. The demand for

fucoidan and sodium alginate in pharmaceuticals, food industries, cosmetics, and health care is enormous (Saji *et al.,* 2022). This demand calls for the exploitation of *E. maxima* for its fucoidan and sodium alginate, which otherwise will end up on beaches, giving off an unpleasant smell.

In conclusion, in this present study, fucoidan and sodium alginate were extracted through a combined and sequential solvent extraction process from the endemic South African seaweed, *Ecklonia maxima*. Fucoidan and sodium alginate from large-scale extractions were compared to optimised SS extractions and commercial standards to determine if there were any differences in their chemical or physical characteristics. The chemical and physical characteristics of the extracts were demonstrated to be very similar to each other and comparable to the commercially available standards. The sequential and combined extraction method for fucoidan and sodium alginate proposed in this study is indeed a feasible and robust approach and can make a significant contribution to the greater seaweed biorefinery and bioeconomy.

The findings of this study highlight the need to scale up the co-extraction process for fucoidan and sodium alginate from *Ecklonia maxima* using larger industrial equipment. Utilising stainless-steel vessels equipped with strong impellers and jacketed temperature control systems will allow for more efficient mixing, flexible temperature control, and improved overall yield on a commercial scale. Industrial trials will be crucial to validate the optimised extraction process's robustness, consistency, and economic feasibility. Working together with industries in pharmaceuticals, nutraceuticals, cosmetics, and food production can facilitate the findings of this study into commercial pipelines, unlocking the full value of *Ecklonia maxima* in South Africa.

Future optimisation of the extraction process should address remaining challenges and concerns, such as the removal of contaminants like proteins and phenolic compounds from fucoidan and improving the separation of fucoidan from sodium alginate residues. Longer delipidation times of additional purification steps could enhance product purity without compromising yields. Advanced analytical techniques such as high-performance size exclusion chromatography and liquid chromatography-mass spectrometry should be employed to characterise the molecular properties of these polysaccharides more accurately. Further investigation using improved experimental designs for ^{1}H-NMR spectroscopy will also allow for a more precise determination of sodium alginate's M/G ratio, correlating these ratios to functional and gelling properties.

Addressing solvent recovery and effluent is another critical step towards the commercial viability of this process. Ethanol consumption will scale significantly with industrial production, requiring improved fractional distillation systems to recover and recycle solvents efficiently, reducing both costs and environmental impact. Solvent effluents, such as spent HCl should also be tested for marine life safety, as the pH can affect the marine life when discharged into the sea. Implementing a life-cycle analysis can further assess the environmental footprint, ensuring that the process aligns with sustainability goals. Additionally, greener extraction methods, such as enzyme-assisted or ultrasound-assisted processes, should be explored to minimise energy usage and waste. Residual seaweed biomass post-extraction also presents opportunities for valorisation into biofuels, compost, or animal feed, supporting a circular economy.

The commercial potential of fucoidan and sodium alginate is vast, given their diverse applications. Sodium alginate, with its high yield of 58.7% (w/w), can be utilised in pharmaceuticals, such as wound dressings, controlled drug delivery systems, and biomaterials. With its bioactivities as an anti-cancer, anti-inflammatory, and anti-coagulant compound, Fucoidan can serve as a natural alternative to heparin, addressing associated side effects like heparin-induced thrombocytopenia. In addition to healthcare applications, fucoidan and sodium alginate are valuable ingredients in nutraceuticals, functional foods, and cosmetics, where they are used as bioactive compounds, thickeners, and hydrating agents.

E.maxima can be positioned as a significant resource in a commercial biorefinery model to meet the increasing global demand for these biopolymers. This would ensure the full utilisation of seaweed biomass by producing multiple value-added products while addressing the current underutilisation of *Ecklonia maxima* globally. Pilot-scale studies will be essential to assess the scalability and market impact of the process, particularly as demand for alginates and fucoidans continues to grow in pharmaceuticals, healthcare, and sustainable biomaterials. By aligning production with regulatory standards such as FDA requirements, *Ecklonia maxima* extracts may emerge as competitive and sustainable alternatives in global markets.

In summary, future studies should focus on industrial-scale validation, process optimisation, solvent recovery, and sustainable practices to enhance the commercial viability of *Ecklonia maxima* extracts. Further research into the molecular characterisation of fucoidan and sodium alginate, focusing on their structural composition, functional properties, and biological activities,

is crucial for understanding their mechanisms of action and optimising their use. This could lead to exploring innovative applications across various fields, such as advanced drug delivery systems, regenerative medicine, and sustainable cosmetic formulations. Addressing the demands of diverse industries, ranging from pharmaceuticals, where their bioactivity offers therapeutic benefit, to cosmetics, where their natural properties enhance product efficacy. These bioactive compounds have the potential to transform multiple sectors. Furthermore, such advancements contribute significantly to the growth of the seaweed bioeconomy, promoting sustainability, creating economic opportunities in coastal regions, and driving innovation in natural, renewable resources.

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Appendices

Appendix A – List of reagents

Table A.1 Names of the reagents utilised and their suppliers.

Name of reagent	Name of Supplier (Catalogue Number)
α-Amylase porcine pancreatic	Megazyme™ (E-PANAA-9G)
α-Glucosidase from *Saccharomyces cerevisiae*	Sigma-Aldrich (029M4179V)
D-Xylose kit	Megazyme™ (K-XYLOSE)
D-Glucuronic acid kit	Megazyme™ (K-URONIC)
D-Mannose, D-Fructose & D-Glucose kit	Megazyme™ (K-MANGL)
L-Fucose	Sigma (F2252)
L-Fucose kit	Megazyme™ (K-FUCOSE)
p-nitrophenol	Sigma (42,575-3)
p-nitrophenyl-α-D-glucopyranoside	Sigma (N1377)
2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl)amino]-D-glucose (2-NBDG)	Sigma-Aldrich (72987)
3,5-Dinitrosalicylic acid	Sigma (D0550)
Acetone	MERCK (8.22251.2500)
Barium chloride dihydrate	Sigma-Aldrich (217565)
Bovine serum albumin (BSA)	Sigma (A7906)
Bradford reagent	Sigma (B6916)
Calcium chloride	Saarchem (1524920EM)
Cabazole	Merck (C5132)
D_2O	Sigma-Aldrich (151882)
Ethanol	MERCK (8.18700)
Folin-Ciocalteu reagent	Sigma-Aldrich (F9252)
Formic acid	MERCK (1038392)
Fucoidan from *F. vesiculosus*	Sigma-Aldrich (F5631)
Gelatine	Fluka (48723)
GOPOD	Megazyme™ (K-GLUC)
Hydrochloric acid	MERCK (1047705)
Lactose and D-Galactose kit	Megazyme™ (K-LACGAR)
Methanol	Sigma-Aldrich (34860)
Phosphate buffered saline	Sigma-Aldrich (P4417)
Phenol	Sigma-Aldrich (P1037)
Sodium alginate	Sigma-Aldrich (W201502)
Sodium carbonate	Sigma-Aldrich (223484)
Sodium hydroxide	MERCK (1.06469.1000)
Sodium sulphate	Saarchem (8525200EM)
Trichloroacetic acid	Sigma-Aldrich (T9159)

Appendix B – Standard curves

Appendix B.1 – Protein Standard Curve

The protein standard curve was generated according to the Bradford protein assay (Bradford, 1976) using BSA standards of concentration in the range of 0 – 0.6 mg/ml.

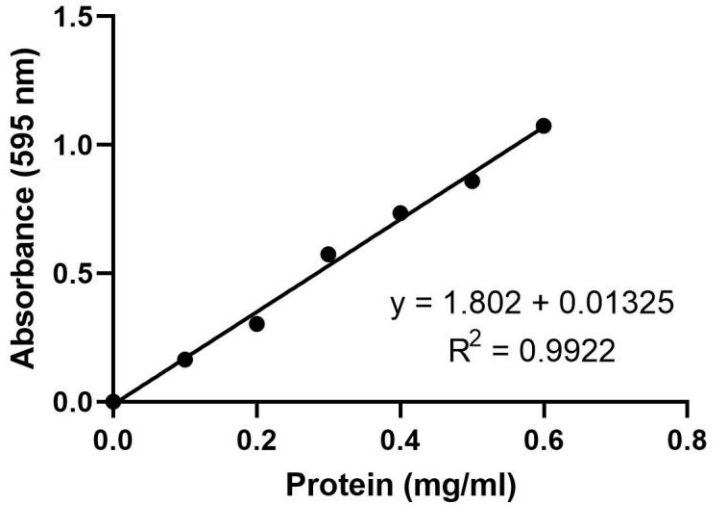

Figure B.1: BSA standard curve. Data points are presented as mean values ± SD (n=3)

Appendix B.2 Phenolics Standard Curve

The standard curve for the phenolic content estimation was constructed according to the Folin-Ciocalteu method using gallic acid (0 -1 mg/ml) as the standard.

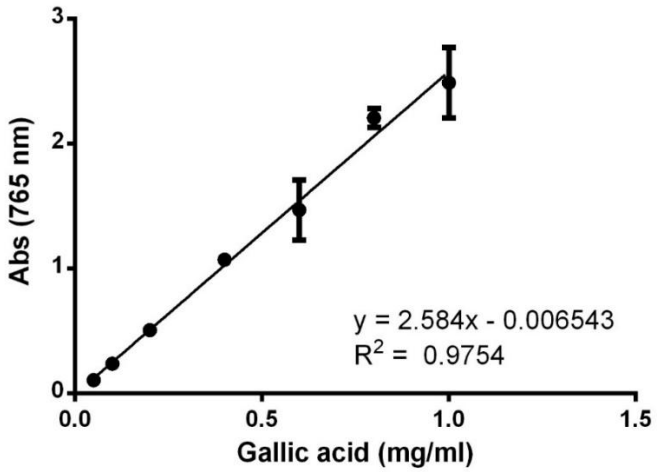

Figure B.2: Phenolics standard curve. Data points are presented as mean values ± SD (n=3).

Appendix B.3 – Gelatine-Barium standard Curve

The gelatin-barium standard curve was constructed according to the modified method of Dodgson (1961), using sodium sulphate ranging from 0 – 1 mg/ml.

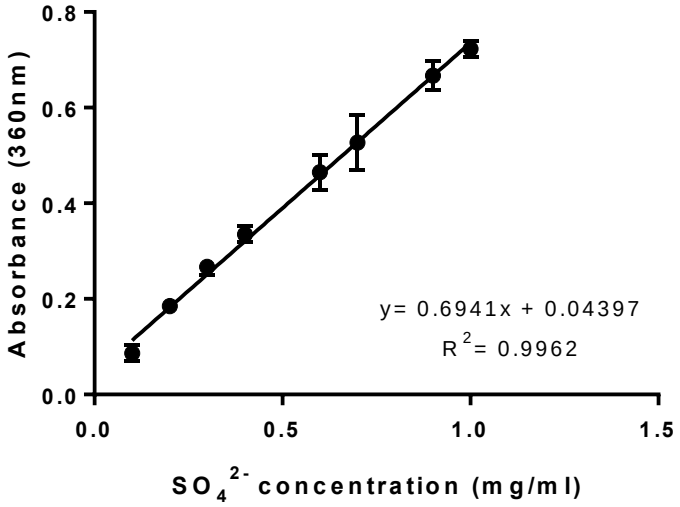

Figure B.3 Gelatin-barium standard curve. Data points are presented as mean values ± SD (n=3).

Appendix B.4 - GOPOD Glucose Standard Curve

The standard curve for the GOPOD was constructed according to the glucose oxidase/peroxidase (GOPOD) method using glucose (0 -1 mg/ml) as the standard.

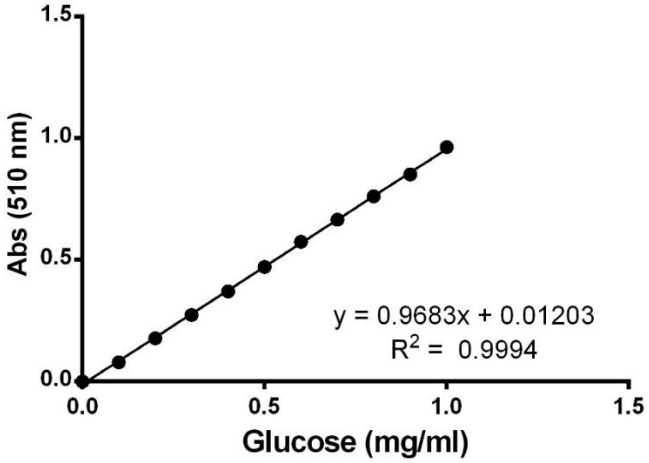

Figure B.4 GOPOD glucose standard curve. Data points are presented as mean values ± SD (n=3).

Appendix B.5 – Phenol-Sulfuric Acid Standard Curve

The phenol-sulfuric acid standard curve was generated according to the phenol-sulfuric acid method using L-fucose (0.1-1 mg/ml) as the standard for fucoidan and using commercial sodium alginate (0.0-1 mg/ml) as the standard for sodium alginate.

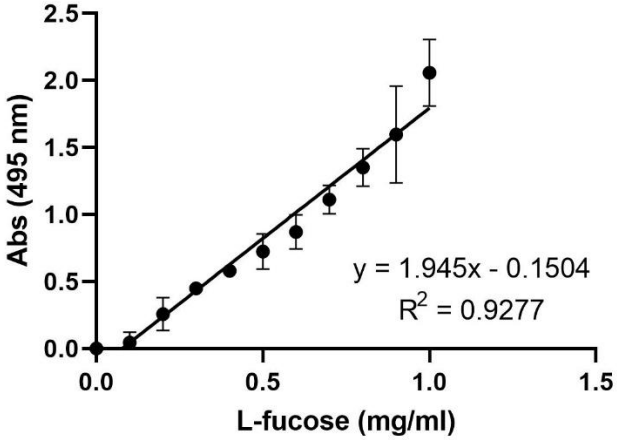

Figure B.5.1 – Phenol-sulfuric acid standard curve for fucoidan quantification. Data points are represented as mean values ± SD (n=3).

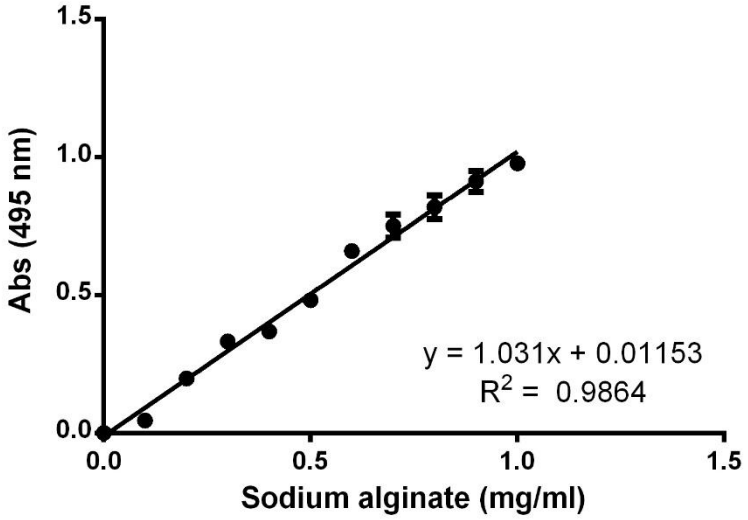

Figure B.5.2 – Phenol-sulfuric acid standard curve for sodium alginate quantification. Data points are presented as mean values ± SD (n=3).

Appendix B.6 - Formula used for Pearson's correlation

$$= \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}}$$