Exploring the potential of polysaccharides from brown seaweed of the coast of Goa in the food industry.

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, **"Exploring the potential of polysaccharides from brown seaweed of the coast of Goa in the food industry"** is based on the results of investigations carried out by me in the Discipline Biotechnology at the School of Biological Sciences and Biotechnology, Goa University under the Supervision of Dr. Samantha Fernandes D'Mello and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Exploring the potential of polysaccharides from brown seaweed of the coast of Goa in the food industry"** is a bonafide work carried out by Ms. Cleona Johanna Lucinda Clotilda De Nazareth under my supervision in partial fulfilment of the requirements for the award of the degree of **Masters of Science** in the Discipline of Biotechnology at the School of Biological Sciences and Biotechnology, Goa University.

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ABBREVIATIONS

µg- Micrograms	mm- Millimeter
A- Absorbance	N- Normal
Ba- Barium	Na- Sodium
C- Celsius	Na_2CO_3- Sodium Carbonate
Ca- Calcium	NaOH- Sodium Hydroxide
$CaCl_2$- Calcium Cloride	nm- Nanometer
cm- Centimetre	OD- Optical Density
CO_2- Carbon Dioxide	P- Phosphorous
DNSA- 3,5- Dinitrosalicyclic Acid	PA- Polyamide
	PBS- Polybutylene Succinate
DPPH- 2,2- diphenyl-1-picrylhydrazyl	PCL- Polycaprolactone
	PE- Polyethene
EAB- Elongation At Break	PHA- Poly-3-hydroxybutyrate
EDX- Energy Dispersive X-ray Spectroscopy	PLA- Polylactic Acid
	PP- Polypropylene
FCC- Food Chemical Codex	R.T.- Room Temperature
$FeCl_3$- Iron chloride	rpm- Revolutions per minute
FTIR- Fourier Transform Infrared	SEM- Scanning Electron Microscope
g- Grams	sp – Species
G- β-L-guluronic acid	SPC- Soy Protein Concentrate
HCl- Hydrochloric Acid	SPI- Soy Protein Isolate
h- Hour	Sr- Strontium

I- Iodine K- Potassium km- Kilometre M- Molar M- α-D-mannuronic acid Mg- Magnesium mg- Milligrams Min- Minute ml- Milliliter	STD DEV- Standard Deviation TPS- Thermoplastic Starch TS- Tensile Strength UV- Ultra Violet W- Weight

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INTRODUCTION

Goa lies along the west coast of India and is part of the Konkan coast and its coastline covers a distance of 120 km. Estuaries, beaches, rocky shorelines, cliffs, bays, and creaks divide it into different regions. Seaweeds grow in the intertidal zone, outside the tidal region; where the high tide spray zone occurs and below the tidal area (in the subtidal zone on submerged reefs and rock boulders) and their growth depends on two main factors, the season and the wave action. *Sargassum cinctum, Sargassum cinereum, Sargassum vulgare, Iyengaria stellata, Dictyota dichotoma, Dictyota bartayresiana, Canistrocarpus magneanus, Padina australis, Padina gymnospora* etc. are a few of the seaweeds recorded (Pereira, 2012). The most common brown seaweeds found in Goa are of the genus *Sargassum, Dictyota* and *Padina* (Naqvi, 1975).

Seaweeds being plants of unique structure and biochemical composition, are used for their versatile qualities in the form of food, energy, medicine, cosmetics, fertilisers, fodder etc. (Dhargalkar & Pereira, 2005) .

In the food industry, there are already several potential economic uses for seaweeds, such as flavor (reduce added salt taste, provide umami flavor) or texture enhancement, nutritional contribution (presence of fibers, minerals, bioactive compounds), bulking agent, sustainability aspects or edible wrapping (Blikra, et al., 2021).

Seaweeds themselves also serve as a source of chemicals (polysaccharides) like agar-agar, carrageenan, and alginates (Pal, 2014). The most prevalent polysaccharide in brown algae is alginate. Alginate is a linear anionic polysaccharide made of α-(1-4) linked molecules of α-D-mannuronic acid (M) and β-L-guluronic acid (G) residues organized

in irregular block wise patterns (G, M, and MG blocks) (Fathiraja, 2022). This primary structural polysaccharide provides flexibility and mechanical resistance to the water's force in the marine habitat where the seaweed grows and is found in both the cell walls and the intercellular matrix of brown seaweed (Rashedy, 2021) They are naturally bound to all of the seawater's salts, especially the Ca^{2+}, Na^{+}, Mg^{2+}, Sr^{2+}, and Ba^{2+} ions (Fertah, 2017). Alginate has special colloidal qualities that are non-toxic, including thickening, stabilizing, gel-producing, film-forming and emulsion-stabilizing abilities (Costa, 2018) because of which, they are used in the production of dairy, bread, meat, and other products or to provide frozen desserts, ice cream alternatives, chocolate milk suspensions, and drinks with a smooth texture (Abraham, et al., 2018). These same properties of alginate have relevance in other industries like the pharmaceutical industry where, they are used as emulsifiers in cosmetics, binders in tablets, mold for dental impressions etc. (Gacesa, 1988). Alginates are also used as immune stimulatory agents (Abraham, 2018), Anti-ulcer agents and Anti-acid compounds (Gacesa, 1988). Seaweed polysaccharides can also be employed as binding agents or as a biomaterial for the creation of bioplastics (Lim, 2021).

Plastics have become very important materials in our lives. Because of their properties such as the ability, to produce molecules with a higher molecular weight, lower reactivity and longer durability by chemically modifying their structure to a variety of strengths and forms, they have been observed to show better performance than metal and wood in certain circumstances. They are reliable and affordable for everyone. Today they

are widely used in several different industries such as textiles, electronics, healthcare, toys, packaging etc. (Gill, 2014). The way that plastic is currently utilized and disposed of significantly pollutes both terrestrial and marine ecosystems. According to estimates, the ocean contains about 250,000 tons of floating plastic, which has a negative impact on marine life and humans by getting into the food chain. Additionally, it has been suggested that the widespread use of plastic in agriculture is a significant cause of soil deterioration and soil microplastics (Heidbreder et al., 2019). One of the main contributors to global warming is the production of toxic substances like dioxins, which are released from plastics (Thiruchelvi et al., 2021).

A viable solution to the world's plastic related issues is bioplastics. Bioplastics can be easily degraded through the enzymatic activities of microorganisms and can be disposed of in the environment. Biodegradable plastics break down into a variety of easily disposed-of natural components, including carbon dioxide, methane, water, biomass, humic matter, and others (Gill, 2014). Hence, using biodegradable plastics is encouraged, particularly if they are created from renewable natural resources. The utilization of biodegradable plastics has numerous advantages. For instance, they use less energy during manufacture (Tsang , et al., 2019), are non-toxic, generate less waste or require less room to manage waste, consume less fossil fuel, and emit fewer greenhouse gases. Biobased polymers can be produced using a variety of techniques and ingredients. Bioplastic is typically made using lipids, proteins, and polysaccharides. Different bioplastics can be used in

various areas due to their unique features and properties. The fact that bioplastics are made from renewable resources is its primary key differentiator.

A variety of biomaterials, including corn, potatoes, vegetable oils, wood, food waste, cereal crops, and others, are utilized to create bioplastics. Nowadays, starch-based bioplastics are the most common, followed by those made of polylactic acid (PLA), poly-3-hydroxybutyrate (PHB), polyamide 11 (PA 11), and organic polyethene (PE). Bioplastics derived from seaweeds are the most recent innovation with a promising future (Lim, 2021). Seaweeds have more promise because of their higher yield, low cost, ease of cultivation in a natural setting, ability to grow in a variety of conditions, and year-round harvestability (Thiruchelvi, 2021). Studies have been conducted to create bioplastics from seaweed. In the soil, seaweed bioplastics decompose within four to six weeks. Furthermore, unlike conventional plastics, which disintegrate into microplastics and are difficult to gather and are not visible to the naked eye, seaweed bioplastics do not form microplastics (Lim, 2021).

Through this experiment, we explore the potential of the brown seaweed from the coast of Goa to provide a cheap source of Sodium Alginate and bioplastic for the food industry.

REVIEW OF LITERATURE

2.1 Seaweeds

The Earth's surface is approximately 70% covered by an expanse of seawater. Marine vegetation is thought to be more primal and abundant than land-based vegetation, and oceans host floating forests with a variety of sea plants and animals. Seaweeds are valuable marine resources that play a significant part in sustaining the sea's abundant biodiversity. Seaweeds are macroalgae that are typically found in marine areas. They are multicellular structures that can be seen with the naked eye and differ from higher plants in that they do not have real roots, stalks, or leaves, making them thalloid in nature. Despite having an attachment organ, such as a holdfast or stipe, that functions similarly to a root or stem, some large seaweeds have distinct origins and tissue systems. The geographical distribution of seaweeds is influenced by a variety of physical, chemical, and biological factors, including the substrate, temperature, light quality and quantity, dynamic tidal activity, winds, and storms, as well as herbivores, microbes, endophytes, symbionts, parasites, and diseases. Seaweeds form the foundation of marine food webs and are a primary source of nutrition for fish and sea urchins. In addition, they offer fish, insects, birds, and mammals' shelter and breeding sites. The most common benthic creatures are seaweeds and coral animals, and their relative abundance is frequently employed as a gauge of ecosystem health (Baweja et al., 2016). Gujarat in India's west coast has a more varied seaweed flora than Maharashtra, Karnataka, and Kerela. Pereira (2012) claimed a total of 146 seaweed species in his investigation, even though Dhargalkar and Agadi (1986) had previously recorded 74 seaweed species. Of these, 64 species belong to red seaweed, 41 to green seaweed and 40 to brown seaweed. Based on their colour, seaweeds can be classified into the Chlorophyta, Rhodophyta, and Phaeophyta families. Green seaweeds called Chlorophyta have chlorophyll a and b as their principal pigment. Red seaweeds

belonging to the Rhodophyta class predominately contain phycobiliprotein. The third is Phaeophyta, the brown seaweed that mainly comprises fucoxanthin. Being aquatic beings, seaweeds have a large percentage of water in their bodies—up to 85%—while the remainder is primarily made up of organic matter and minerals. The dry matter of seaweeds is made up of total lipids ranging from 0.5 to 3.5%, proteins ranging from 3 to 50%, total carbohydrate content ranging from 21 to 61%, and minerals ranging from 12 to 46%. In comparison to terrestrial biomass, seaweed has a higher growth rate and requires fewer resources for production (no land, freshwater, fertilisers, or pesticides are needed) (Baghel et al., 2021). Seaweeds are not only inexpensive, widely accessible, and sustainable, but they can also absorb CO2 during photosynthesis, decrease ocean acidity, and decrease global warming (Lim et al., 2021). Seaweeds have a wide range of uses, including those for paper, plastic, food, cosmetics, fertiliser, biofuel, wastewater treatment, and more (Lim et al., 2021). The primary commercial items typically derived from seaweed biomass are polysaccharides like agar, carrageenan, and alginate. Seaweeds that include agar are called agarophytes, those that contain carrageenan are called carrageenophytes, and those that contain alginate are called alginophytes. These hydrocolloids are widely utilised as an ingredient in a variety of industries, including the manufacturing of bioplastics, food, beverages, dairy, animal feed, cosmetics, personal care, pharmaceuticals, healthcare, textiles, printing, and paper coating. Additionally, considerable levels of storage sulphated polysaccharides like ulvan and porphyran are present in the members of the genera *Ulva* and *Porphyra.* Such polysaccharides have the potential to be used to make medicines and nutraceuticals due to their wide range of biological activity. The minerals derived from seaweed are yet another element used in fertiliser on a commercial scale. In

addition to this, seaweeds have little to no lignin, which makes it easier to recover pure, undamaged cellulose, which is more suitable for biomedical applications (Baghel et al., 2021).

2.2 Brown seaweeds

Brown algae are the largest category among the three main types of seaweed in terms of thallus size and total biomass generated. Brown seaweeds are the dominating taxa of the marine littoral zone from the subpolar to the equatorial areas and exhibit remarkable morphological variation. The pigment fucoxanthin, which gives all brown seaweeds their brown colour, and laminarin, which serves as their primary food source for storage, distinguish them as members of the Phaeophyceae family. Many of the filamentous, smaller species of brown seaweed are epiphytes, whereas the majority of them are lithophytes, which need a solid, firm surface to cling to. In cooler seas, brown seaweeds predominate in the upper littoral region and intertidal zone. The cell wall is often gelatinous and has two layers: an inner layer formed of cellulose, and an outer layer primarily made of algin and fucoidan, along with amorphous mucilaginous matrix fraction and mucilaginous alginates. The cell walls of brown seaweeds also contain phlorotannin's, which are halogenated, sulphated phenolic chemicals. Different regions of the same thallus as well as different species and seasons exhibit dramatically varying relative abundances of the cell wall constituents. In brown algae, the usual weight ratios of alginates, fucoidans, and cellulose are 3:1:1. (Baweja et al., 2016). Brown seaweeds are popularly used in the food, fertilizer, and pharmaceutical industries. Some examples of brown seaweeds are *Saccharina sp., Himanthalia sp., Laminaria sp., Ascophyllum sp., Undaria sp., and Sargassum* sp. (Lim, et al., 2021).

2.3 Polysaccharides found in Brown seaweeds.

Since polysaccharides are non-toxic, type of natural biopolymer component found in practically all biomaterials, they have garnered a lot of attention. Polysaccharides have a wide range of possible biological effects, primarily anticancer, antibacterial, antiviral, hyperglycaemic, antioxidant, and anti-inflammatory ones. The main sources of these polysaccharides include plants, mushrooms, bacteria, algae, and mammals. Algal bio-polysaccharides, among other bioactive compounds, are currently drawing attention from all over the world due to their promising biomaterial and robust biological abilities (Gopu et al., 2020).

Laminaran, fucoidan, and alginate make up the majority of the brown seaweed polysaccharides, with smaller amounts of cellulose, mannitol, sargassan, and other substances. However, only three components of brown seaweeds—alginate, fucoidan, and cellulose—are used to make bioplastic, while there are still undiscovered polysaccharides and many studies concentrate on Sargassum species. The primary component of sulphated fucoidan is -L-fucose, with minor amounts of galactose, mannose, xylose, glucose, and glucuronic acids. Depending on the season, environment, and method of extraction, fucoidans may support a total of 2 to 20% of the dry weight of brown seaweed. Alginates are anionic linear polysaccharides that make up to 40% of the dry weight of brown seaweeds and have been shown to have the ability to produce edible films. Alginates are alginic acid salts made up of 1,4-linked monomer units of β-D-mannuronic acid (M) and α-L-guluronic acid (G). While G type alginates create irregular conformation chains, M type alginates form linear chains. Alginates can quickly cross-link with a divalent cation through ion exchange, typically using calcium chloride, or by evaporating the solvent (Lim et al., 2021).

Alginates that are suitable for industrial application include propylene glycol alginate, sodium, potassium, ammonium, mixed sodium-calcium, and mixed sodium-ammonium salts of alginic acid. The molecular weight, calcium content, particle morphology (granular or fibrous), particle size distribution, and the mannuronic acid: guluronic acid ratio of these water-soluble alginates vary depending on the form in which they are formed (Glicksman, 1987).

Alginate possesses special colloidal qualities, including thickening, stabilising, suspending, film-forming, gel-producing, and emulsion stabilising, making it an ideal hydrocolloid to use in the food industry. (Costa, et al., 2012).

2.4 Application of seaweeds in the food industry.

Seaweeds contain the highest concentration of natural antioxidants and antimicrobials. They are also a great source of minerals like Ca, P, Na, K, and I, as well as vitamins like A, Bl, B12, C, D, and E, riboflavin, niacin, and folic acid (Gupta & Abu-Ghannam, 2011). Since the beginning of time, various seaweeds have been consumed by humans as food in many nations, including Costa Rica, Japan, China, and Egypt. Due to its abundance of proteins and vitamins, the red seaweed *Porphyra* (Nori) is used to make sushi in many nations, including Japan, South Korea, the United States, and the United Kingdom (Baghel et al., 2021). Seaweeds are consumed fresh as a salad in Malaysia and Indonesia. Seaweeds are largely employed in the commercial manufacturing of additives for both food and non-food uses (such as alginates) in the European Union. (Tiwari & Troy, 2015)

Hydrocolloids produced by seaweed are found in the intercellular and cell walls. The brown algae (Phaeophyceae) generate uronates (alginates), while members of the red algae (Rhodophyta) create galactan (such as carrageenans and agars).

Carrageenans are natural compounds that have been used for decades in food applications and are widely recognised as safe; they are one of the main texturizing agents utilised by the food industry (Pereira et al., 2009).

The thermoreversible gelling agents κ- and L-carrageenans are both used extensively in water and milk gelling systems, including in dessert gels, jellies and jams, fish gels, pet meals, puddings, flans, frozen desserts and many other items. In numerous thickenings, suspending, and bodying applications, such as milk shakes, flavoured milk, beverage mixes, syrups, sauces, and related goods, α-carrageenan, the non-gelling carrageenan, is utilised. Alginates are mostly utilised in

i. Frozen desserts like ice creams to provide it with a smooth consistency by controlling the growth of ice crystals
ii. Dairy products to stabilise goods like cream cheese, whipped cream and other cultured dairy goods
iii. Bakery goods to stabilise icings, toppings, meringues and fillings for chiffon or fruit pies and to stop food from clinging to packaging
iv. Drinks where they are used to stabilise milkshakes, the foam in beers and to sustain the fruit pulp in some non-carbonated fruit beverages
v. Dessert gels to set dessert puddings quickly and to allow custards with milk or milk solids to stay soft without developing hard skin.
vi. Manufactured meals as a matrix gel in manufactured foods like meat pieces and onion rings made from freshly sliced onions.

Propylene glycol alginate works great as a thickening and stabiliser in salad dressings and is also used to stabilise a variety of gravies, meat sauces, and barbecue sauces. Fish that has been quickly frozen can be given a sodium alginate-

calcium chloride treatment to produce a sturdy, freeze-thaw resilient coating that extends the shelf life of the fish by keeping out air (Glicksman, 1987). Bioplastics derived from seaweeds are the most recent innovation with a promising future. (Lim et al., 2021)

2.5 Bioplastics

Galalith and Polyhydroxyalkanoates (PHAs) are two of the first bioplastics, having been identified almost a century ago. The market currently offers a wide variety of bioplastics, including but not limited to Mirel, Bio-PET, Poly(butylene adipate-co-terephthalate), Polylactic Acid (PLA), and Poly(butylene adipate-co-terephthalate). The market is now dominated by starch-based polymers like blends with polycaprolactone (PCL) and polybutylene succinate (PBS). Although a bioplastic's definition is not standardised, the most frequently recognised definition is that a bioplastic is any polymer that is either biobased, biodegradable, or both (Nandakumar et al., 2021). Polymers made from renewable resources or the agro-industrial and marine by-products and wastes are some sources for bioplastics. For example, Soy protein is taken from the same soybeans that are used to make soy oil. Soy flour is produced as a by-product of this process, and it can be processed to produce soy protein isolate (SPI) and soy protein concentrate (SPC), which adds value to agricultural by products (Leceta et al., 2014).

The different types of bioplastics are:

i. Starch-Based Bioplastics - Starch-based polymers are defined as those that contain either native or modified starch moieties. This group can include polymers made from the fermentation of starch as well as mixtures of starch and natural or synthetic plastics. Many of the thermoplastics used today are made of this, which makes up

around 50% of the worldwide bioplastics market. Thermoplastic Starch (TPS) and Bio-PET are 2 examples of starch-based bioplastic.

ii. Cellulose-Based Bioplastics: Made from cellulose esters or other cellulose derivatives. Because cellulose comprises glucose molecules linked together by a β (1,4) bond, it must be digested by ruminants using particular symbiotic microorganisms. Examples include cellulose acetate and methyl cellulose.

iii. Aliphatic Polyesters - These polyesters contain components that are more resistant to hydrolytic breakdown. such as PHA and PLA.

iv. Protein-Based Bioplastics- Made of protein are derived from sources including milk, gluten from wheat, and other sources. For example, casein bioplastics.

v. Lignin-Based Bioplastics - Although lignin has long been produced as a by-product of the cellulose industry, it has only recently become a significant biorefinery project. Blends of PP and lignin polymers, blends of PHA and lignin polymers, etc. are a few examples (Duval et al., 2014).

vi. Chitin-Based Bioplastics- N-acetyl-D-glucosamine units are joined by (1,4) bonds to form the biopolymer chitin, which is the second most prevalent biopolymer after cellulose. Although chitin is found in the exoskeletons of arthropods and in the cell walls of yeast and fungi, the shells of crustaceans like crabs, prawns, and shrimps are the main source of its extraction. For instance, bioplastics made of chitosan, chitin blended with PP, etc.

To make bioplastic, these biopolymers can either be directly extracted from the biomass, or polymers can be made from bio-derived intermediates or polymers may be produced by microorganisms. Some applications for starch polymers include the manufacture of products such as toothpicks, food service ware, and planting pots. Starch-based polymers can be combined with other biodegradable plastics to create

bioplastics that are both biodegradable and compostable. These bioplastics can be used to create compostable packaging, mulching films, and recycling bags for organic waste. Chitin-based polymers can be used in a variety of industries, including biomedicine, food technology, the textile industry, the manufacturing of nanoparticles, and agriculture because they are biodegradable and biocompatible. Heinz and Ford also worked together in an inventive way, supplying Ford with leftover tomatoes that would have otherwise gone to waste so that it could process them into tomato fibres and utilise those along with other plastics in injection moulding to create automobile parts. Later, the packaging for Heinz ketchup bottles included this technique (Nandakumar et al., 2021).

2.6 Seaweed based bioplastics

Plant-based bioplastics made from starch (maize) are promising but call for the use of land that would otherwise be used for agriculture. Some bacterial species store internal polyhydroxyalkanoate particles as sources of carbon and energy within their cells, providing another source for the generation of bioplastics. Despite this, their uses are constrained by poor biomass yields and challenging cultivation (Farghali et al., 2023).

Seaweeds are certainly more desirable than other biomaterials. We can say this because seaweeds can develop without freshwater, soil, or pesticides. Seaweeds are appropriate for use in the production of polysaccharide films due to their greater polysaccharide content. Recent studies have revealed that raw seaweed called *Kappaphycus alvarezii* can be directly used to create plastics instead of derivatives of seaweed, making it a less expensive and healthier alternative. Different seaweed species can be used or blended with other materials to increase qualities and

performances to solve weaknesses like low water vapor barrier capabilities and mechanical strength. In the soil, seaweed bioplastics decompose within four to six weeks. Furthermore, unlike conventional plastics, seaweed bioplastics do not disintegrate into microplastics. (Lim et al., 2021).

Many polysaccharides from various algae have been employed to create films that have attracted a lot of interest from the packaging sector. They were founded on the distinct colloidal nature, availability, affordability, and good film-forming property. They can produce successful films with good optical quality (transparency), non-toxicity, solubility, good tensile strength (TS), and elongation at break (EAB). They can also produce films that act as a barrier to water vapor, oxygen, and UV light (Guidara, et al., 2020) .

Seaweed films can be utilized for a variety of food packaging, coating, and edible capsule applications, including the packaging of instant noodles flavoring, facial treatments, tea bags, vitamins, and more (Lim et al., 2021). A startup called Nutrafilm says its seaweed food packaging is also edible and was strengthened with chemicals like CaCl2 that have no negative side effects (Nandakumar et al., 2021).

2.7 Why choose seaweeds from the coast of Goa.

Goa lies on the west coast of India and covers a distance of 120 km along its coastline. According to Pereira (2012), 146 species of seaweed are found along this coastal belt of which 40 belong to the Brown Seaweed family. During low tide, the seaweed can be seen accumulated along the shoreline. Thus, the collection of this seaweed and exploring its potential could lead to a more effective use of them.

AIM AND OBJECTIVES

Aim:

To isolate alginate polysaccharide from brown seaweed found on the coast of Goa and explore its potential in the food industry.

Objectives:

* To collect brown seaweeds from the coast of Goa and extract alginate polysaccharide from them.
* Characterization of the extracted polysaccharide.
* To synthesize and characterize a seaweed-based biopolymer film.
* To assess the application of the seaweed-based biopolymer film in the food industry

MATERIALS

AND

METHODS

3.1 Seaweed sampling and processing.

Two species of brown seaweeds were collected from Odxel beach and Vainguinim beach along the coast of Goa during low tide in the post-monsoon season. The samples were identified as *Padina tetrastromatica* and *Sargassum cinereum* based on their morphological characters by Prof. Vijaya Kerkar from Botany Discipline, School of Biological Sciences and Biotechnology, Goa University.

The seaweeds were then washed with tap water to remove any sand or other particles and were sun-dried. Once completely dry, they were stored in the dark at room temperature.

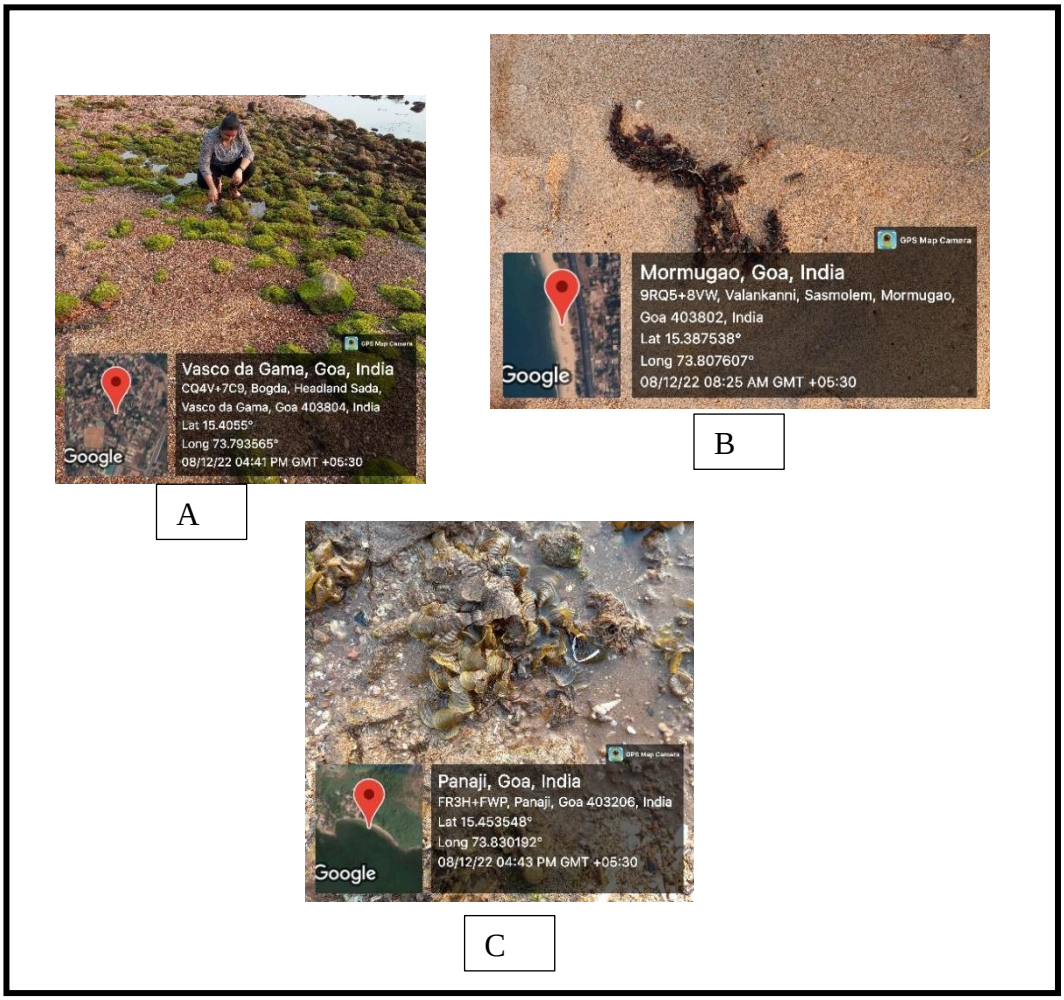

Figure 1: (A) Collection of seaweed samples from the coast of Goa (B) *Sargassum* sp. collected at Baina Beach (C) *Padina* sp. collected at Odxel Beach.

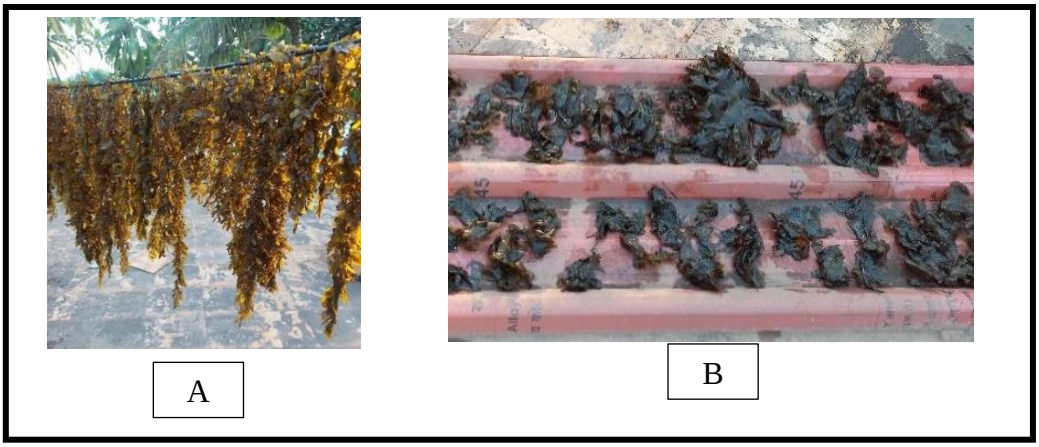

Figure 2: Sun drying of (A) *Sargassum* and (B) *Padina* at room temperature.

Extraction of polysaccharide from seaweed

Extraction of Alginate from Brown Seaweed was carried out using the method in Widiyanti (2012) with a few modifications. Dried seaweed samples (25g) were cut into small pieces and submerged in 1% HCl for 1 h. Excess HCl was discarded and to that 4% Na_2CO_3 was added to submerge the seaweed again. This was heated for 2 h at 60°C with continuous stirring. It was then diluted with distilled water and allowed to stand for 30 min. The mixture was then filtered overnight using Whatman filter paper. The filtrate was collected and the pH was adjusted to 2-3 by adding 5% HCl and then left overnight to form foam lumps. The foam lumps were collected and 1M NaOH (10 mL) was added to it and kept for 10 min. 99% Isopropanol was added at a ratio of 1:2 to the sample and left for 10 min. The mixture was then centrifuged at 5000 rpm at 4°C for 10 min. The pellet was collected and allowed to dry. The alginate yield was measured as follows:

$$Yield\ of\ alginate\ (\%) = \frac{weight\ of\ alginate}{weight\ of\ dried\ seaweed\ biomass} \times 100$$

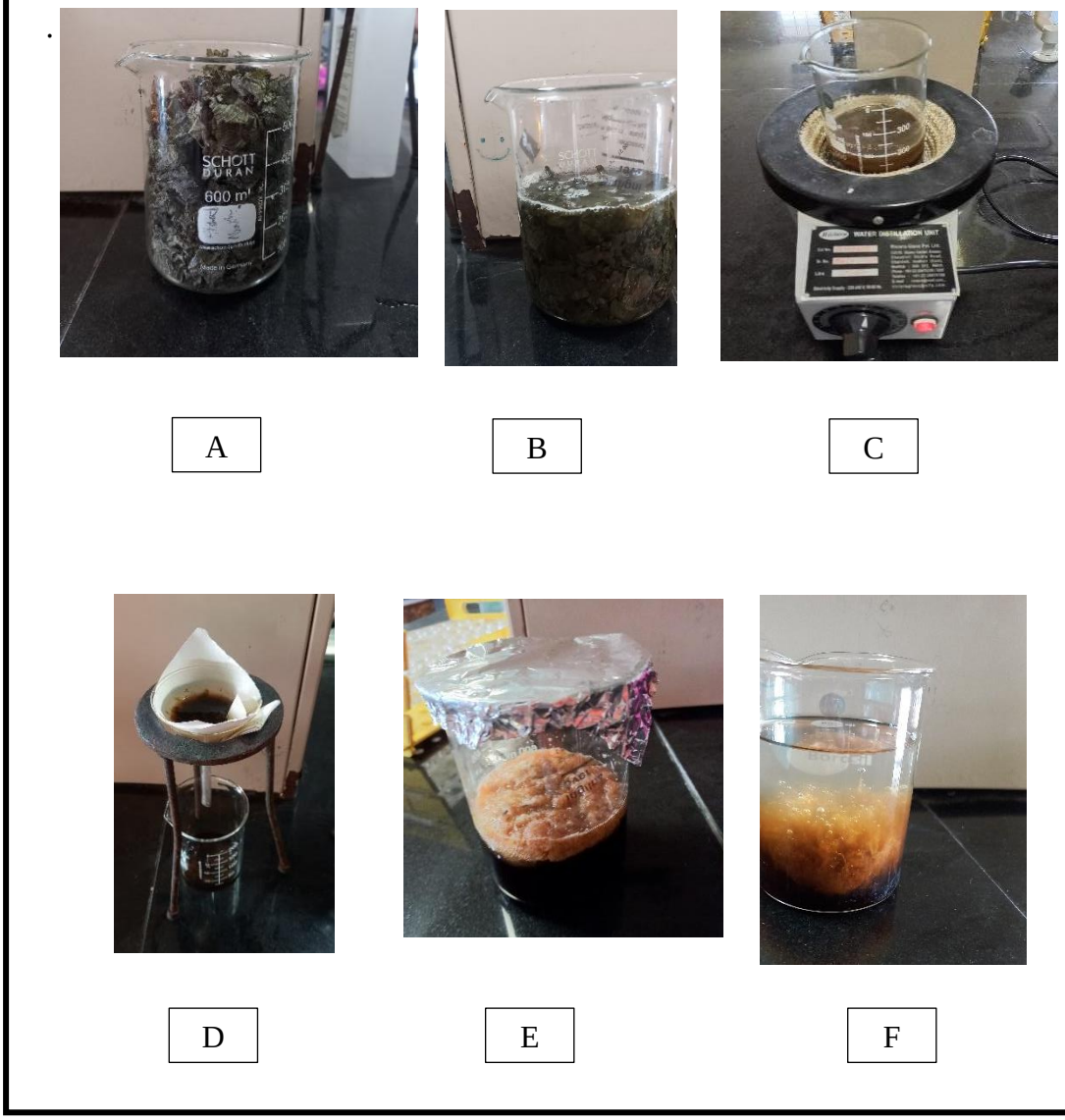

Figure 3: Extraction of Sodium alginate from seaweed (A) Seaweed sample (B) Sample soaked in HCl (C) Sample in 4% Na2CO3 being heated (D) Filtration of solution (E) Formation of lumps after adding HCl (F) Solution on addition of isopropanol.

3.2 Preparation of Alginate Beads

Alginate Beads were prepared following the method stated in Kaur (2018) with few modifications. 1g crude sample of alginate polysaccharide was mixed with 2 mL of distilled water. The mixture was heated till the alginate dissolved. The mixture was then taken up in a syringe and added drop by drop to a beaker containing chilled 2% $CaCl_2$ and observed for the formation of beads. The beads were then, filtered and washed with distilled water and dried on filter paper

Physico-chemical characterization of the extracted Polysaccharide

3.4.1 Estimation of pH and Color

The pH was measured according to the protocol mentioned by (Rashedy et al., 2021) 1g of extracted polysaccharide was weighed and added to 100 mL of distilled water. Once dissolved, the pH was measured. Color was observed visually against a white background.

3.4.2 Estimation of Moisture Content

Moisture content was measured as per the protocol mentioned by Permatasari, et al., (2022) with slight modifications. The extracted polysaccharide (2 g) was weighed and kept for drying in the oven for 20 h at 90 °C. The dried sample was then removed from the oven and allowed to cool before its weight was measured again. The moisture content was calculated as follows:

$$Water\ Content\ (\%) = \frac{W_1 - W_2}{W_1} \times 100$$

Where; W_1 is the weight of the sample before drying.

W_2 is the weight of the sample after drying.

3.4.3 Estimation of Viscosity

Viscosity was measured following the protocol mentioned by Rashedy et al., (2021) with few modifications. A 1% solution of extracted polysaccharide in distilled water was prepared and the viscosity was measured using a viscometer (Borosil Viscometer).

Figure 4: Borosil viscometer used to measure viscosity

3.4.5. Evaluation of Purity of Alginate by Qualitative Phytochemical Analysis

Rapid qualitative analysis was carried out to test for impurities in the extracted polysaccharide.

3.4.5.1 Test for Flavonoids

Presence of flavonoids was tested using the qualitative method mentioned by Rashedy et al., (2021). The extracted polysaccharide (0.5 g) was mixed with 1 mL of dilute NaOH. To this solution 1 mL of 0.1N HCl was added. The presence of flavonoids was indicated by a colour change from yellow to colourless.

3.4.5.2 Test for Alkaloids

Presence of alkaloids was tested using the qualitative method mentioned by Rashedy et al., (2021). 0.5g of extracted polysaccharide was mixed in 10 mL of dilute HCl (0.1N). The solution was then filtered and 3 mL of the filtrate was mixed with 1 mL of 1% HCl. To this, few drops of Meyers reagent were added. Formation of creamy white ppt indicated the presence of Alkaloids.

3.4.5.3 Test for Tannins

The presence of tannins was tested using the qualitative method mentioned in (Rashedy et al (2021). 0.5g of extracted polysaccharide was boiled in 10 ml distilled water and then filtered. To this filtrate, a few drops of 0.1% $FeCl_3$ was added. Appearance of blue black or brownish green color indicates the presence of tannins.

3.4.6 Biochemical Analysis of Alginate Polysaccharide

3.4.6.1 Carbohydrates Analysis

Carbohydrates analysis was carried out using the DNSA method (Miller, 1959). 1 mg of crude polysaccharide was mixed with 2 mL of distilled water. To this solution 2 mL of DNSA reagent was added and kept for incubation in a boiling water bath for 15 min. Absorbance was measured at 540 nm and plotted against a standard graph.

Table 3.1: Carbohydrate Estimation.

Sample	Volume of unknown (mg)	Distilled water (mL)	Volume of DNSA Reagent (mL)	Incubation In boiling water bath for 15 minutes.	OD at 540 nm
Blank	-	2	2		
Padina polysaccharide	1	2	2		
Sargassum polysaccharide	1	2	2		

3.4.6.2 Total Protein Analysis

Protein estimation was carried out using Folin Lowry method (Lowry, Rosebrough, Farr , & Randall, 1951). 1 mg of crude polysaccharide was mixed with 1 mL of distilled water. To this 3 mL of Reagent C was added and kept for incubation at R.T for 10 mins. Then 0.5 mL of Folin Ciocalteu Phenolic Reagent was added and tubes were kept again for incubation at R.T for 30 mins. Absorbance was measured at 660 nm and compared to that of a standard graph.

Table 3.2: Protein Estimation.

Sample	Volume of unknown (mg/ml)	Distilled water (mL)	Reagent C (mL)	Incubate at R.T for 10 mins.	Folin Ciocalteu Phenolic Reagent (mL)	Incubate at room temperature for 30 minutes	OD at 660 nm
Blank	-	1	3		0.5		
Padina polysaccharide	1	1	3		0.5		
Sargassum polysaccharide	1	1	3		0.5		

3.4.7 Antioxidant Analysis from Alginate Polysaccharide

The Antioxidant properties of the crude polysaccharide was measured using the DPPH radical scavenging activity protocol by Blois, (1958) with few modifications. 5 mg of crude polysaccharide was mixed with 2 mL of 70% ethanol. To this, 1 mL of DPPH reagent was added and the tubes were kept for incubation at R.T. in the dark for 30 min. Absorbance was measured at 517 nm and compared to a standard graph.

Table 3.3: Antioxidant Analysis

Sample	**Amount of unknown (mg)**	**70% Ethanol (mL)**	**Volume of DPPH Reagent (mL)**	Incubation at R.T for 30 mins.	OD at 517 nm
Blank	-	2	-		
Control	-	2	2		
Padina polysaccharide	5	2	2		
Sargassum polysaccharide	5	2	2		

3.5 Synthesis of Biopolymer Film.

12.5 g of Alginate was mixed with 45 mL distilled water and heated till the alginate dissolved completely. To this, starch was added in a 1:4 (starch: alginate) ratio. Then, 5 mL of 50 % Glycerol was added. The solution was then brought to a boil and poured into Petri plates and left undisturbed in the oven at 100 °C till dry.

3.6 Characterization of the Biopolymer Film

3.6.1 Physical Properties

3.6.1.1 Thickness

The thickness of the biopolymer film was measured using a screw gauge micrometre (Aerospace Micrometre). 5 random spots were chosen and their thickness was measured (Guidara et al., 2020).

3.6.1.2 Solubility

Solubility was measured as per the method mentioned in Moey et al. (2014) with minor modifications. Biopolymer films were cut into 1 × 1cm pieces and their weight was taken. The pieces of the film were then immersed in 50 mL of distilled water for 30 min at 25 °C on a shaker. After 30 min, the solutions were filtered using Whatman filter paper and kept for drying in the oven at 60 °C overnight. The dry weight was measured. Solubility of the films was calculated as:

$$Solubility\ (\%) = \frac{W_i - W_f}{W_i} \times\ 100$$

Where; W_i is the initial weight of the sample.

W_f is the final weight of the sample after drying.

3.6.1.3 Moisture content

The moisture content was measured according to the method stated in (Oluwasina & Awonyemi, 2021). 2 x 2 cm pieces of biofilm were cut and weighed. They were then dried in the oven at 105° C for 3 h. The final weight was measured again after cooling. Moisture content was calculated as:

$$Water\ Content\ (\%) = \frac{W_1 - W_2}{W_1}\ X\ 100$$

Where; W_1 is the weight of the sample before drying.

W_2 is the weight of the sample after drying.

3.6.2 Transparency

The transparency of the biopolymer film was measured following the protocol from Moey et al., (2014). Film strips were cut to fit the cuvettes. The cut pieces of film were then placed inside the cuvettes and the absorbance was measured at 600 nm. The Transparency was measured as:

$$T = \frac{A_{600}}{X}$$

Where; A_{600} is the absorbance at 600 nm

And X is the thickness of the film.

3.6.3 Morphological and elemental composition of the biopolymer film

The surface morphology and elemental composition of the biopolymer films were observed using scanning electron microscope and energy dispersive X-ray spectroscopy. The samples were cut into 1×1 cm pieces and sputter coated with gold before being subjected to SEM-EDX analysis.

3.6.4 Assessing the Anti-Oxidant potential of the bioplastic

The Antioxidant properties of the biofilm was measured using the DPPH radical scavenging activity protocol by Blois, (1958) with few modifications. 5 mg of bioplastic film was mixed with 2 ml of 70 % ethanol. To this, 1 mL of DPPH reagent was added and the tubes were kept for incubation at R.T in the dark for 30 min. Absorbance was measured at 517 nm and compared to a standard graph prepared using Ascorbic acid.

Table 3.4: Antioxidant Analysis

Sample	**Amount of unknown (mg)**	**70 % Ethanol (mL)**	**Volume of DPPH Reagent (mL)**		
Blank	-	2	-	Incubation at R.T for 30 minutes.	**OD at 517 nm**
Control	-	2	2		
Padina biofilm	5	2	2		
Sargassum biofilm	5	2	2		
Standard biofilm	5	2	2		

3.7 Application of Biopolymer Films in Prevention of Oxidation.

The ability of the biofilm to prevent oxidation was tested. The bioplastic was cut and sealed on 3 sides. Pieces of apple were cut and placed in the packets and sealed completely to form air-tight packets. A control piece of apple was kept exposed to the atmosphere. All samples were kept for incubation at room temperature for 1.5 h. After 1.5 h, the apple pieces were taken and homogenised and filtered. 5 mL of water was added to 1 mL of filtrate and mixed. Absorbance was measured at 475 nm.

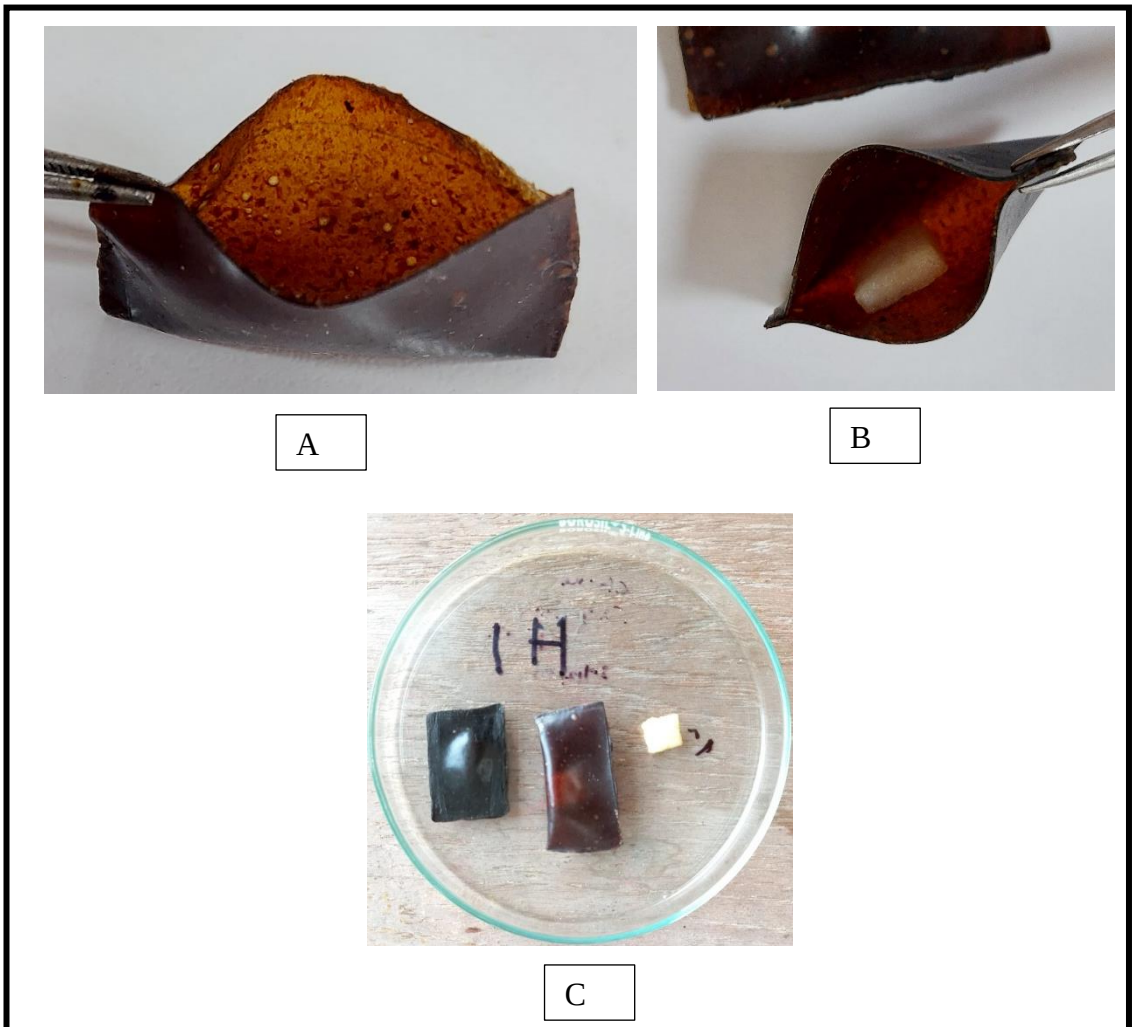

Figure 5: (A) Biofilm packets prepared (B) Apple piece placed inside the packet (C) Biofilm packets kept for incubation.

RESULTS AND DISCUSSION

5.1 Extraction of polysaccharides from seaweed.

Polysaccharide was extracted from brown seaweeds *Padina tetrastromatica* and *Sargassum cinereum* by Hot Alkali Extraction process. The yield from *Padina* sp. was calculated to be 23.6% and from *Sargassum* sp. was calculated to be 38.64%.

There are five processes in the alginate commercial extraction process: acidification, alkaline extraction, solid-liquid separation, precipitation, and drying. The primary step is alkaline extraction since it corresponds to the actual extraction phase. When seaweed is exposed to sodium carbonate solution after being acidified, insoluble alginic acid is transformed into soluble sodium alginate, which enters the aqueous phase (Abraham et al., 2018). During extraction, alginic acid salts are initially changed into free alginic acid through proton exchange with a strong acid (like HCl). The following stage involves neutralizing insoluble alginic acid with an alkali to create water-soluble sodium alginate, which is then retrieved from the extraction solution by precipitating with hydrochloric acid, calcium chloride, or alcohol before being dried and ground (Alba & Kontogiorgos, 2018).

According to a study by Rhein-Knudsen et al., (2017) the yield of *Padina* was 46.15 % and of *Sargassum* was 86.37 %. In another study by Faidi et al., (2020) the yield from *Padina* was 28.7% and the yield of *Sargassum* was 16.9% in a study by Torres, et al., (2007). On comparison, the extracted polysaccharide yield of *Sargassum* was greater than that seen by Torres, et al., but was considerably lower than the one seen by Rhein-Knudsen et al. The extracted polysachharide yield from *Padina* was much lower than the yield shown by Rhein-Knudsen et al. but much closer to that seen by Faidi et al. The amount of alginate that can be extracted varies on the age, species, and environmental factors employed, including light intensity,

water temperature, currents, and nutritional state. It also depends on the extraction methods used (Rashedy et al., 2021).

5.2 Confirmatory test for Alginate

Crude polysaccharide solution on contact with cold $CaCl_2$ showed formation of bead like structures. This indicated that the polysaccharide was sodium alginate.

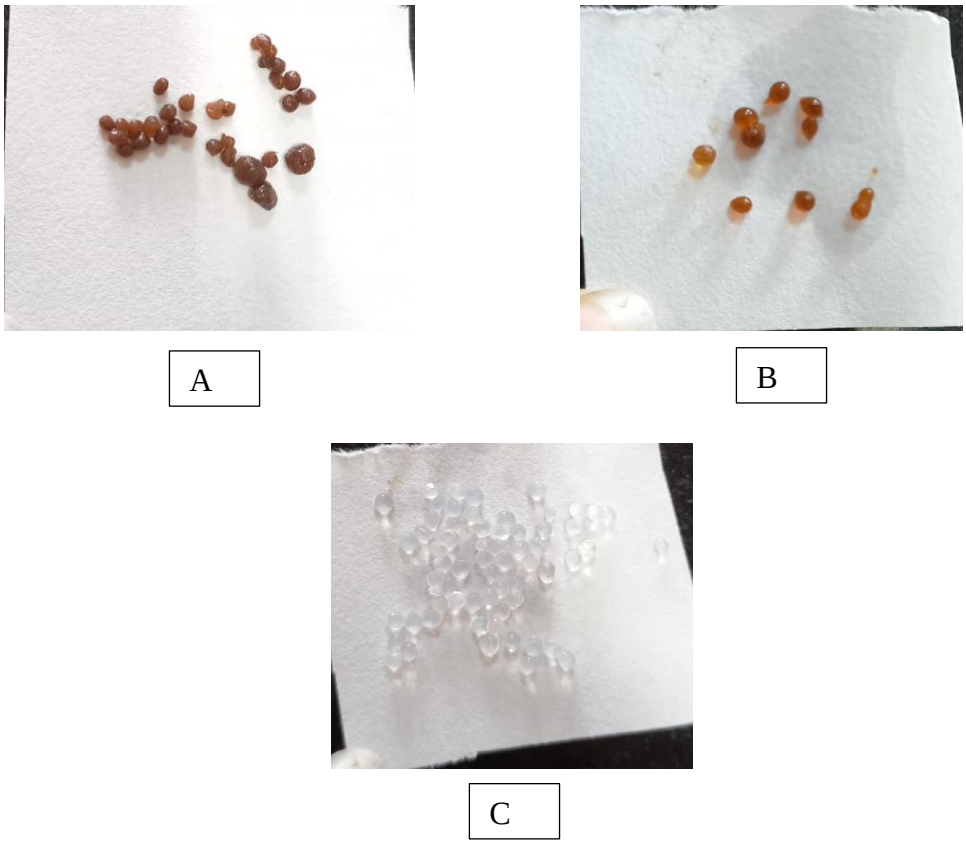

Figure 6: (A) Ca-Alginate beads from Polysaccharide extracted from *Padina* sp. (B) Ca-Alginate beads from Polysaccharide extracted from *Sargassum* sp. (C) Ca-Alginate beads from lab grade Standard Alginate.

Liquid alginate solutions are instantly converted into gel on contact with Ca^{2+} polycations. This occurs due to binding between guluronic acid blocks in alginate and Ca ions (Sugiura, et al., 2005).

5.3 Physicochemical characterization of Alginate Polysaccharide

5.3.1 Estimation of pH and Color

5.3.1.1 pH

The pH was measured using Eutech Instruments pH 700 device and was recorded as seen in Table 5.1

Table 5.1: pH Estimation

Sample	**pH**
Padina alginate	9.97 ± 0.05
Sargassum alginate	4.76 ± 0.03

According to a study by Rashedy et al.,(2021), *Padina* sp. and *Sargassum* sp. showed a pH of 9.72 and 9.76 respectively whereas Mokoginta et al., (2019) stated that *Sargassum* sp. showed a pH of 9.4.

While, our polysaccharide extracted from *Padina* sp. showed similar pH to those stated by previous research, *Sargassum* sp. showed a much lower pH. However, the Food Chemical Codex (FCC) has recognised that the food quality of sodium alginate in the food sector needs to be between pH 3.5-10. Thus making the extracted alginate suitable for use in the food industry.

5.3.1.2 Color

The extracted polysaccharides from *Padina tetrastromatica* and *Sargassum cinereum* was found to be brown in color.

According to a study by Rashedy et al.,(2021), *Padina* sp. and *Sargassum* species showed a pale yellow color whereas we obtained brown color polysaccharide. This could be due to bleaching carried out by Rashedy et al.

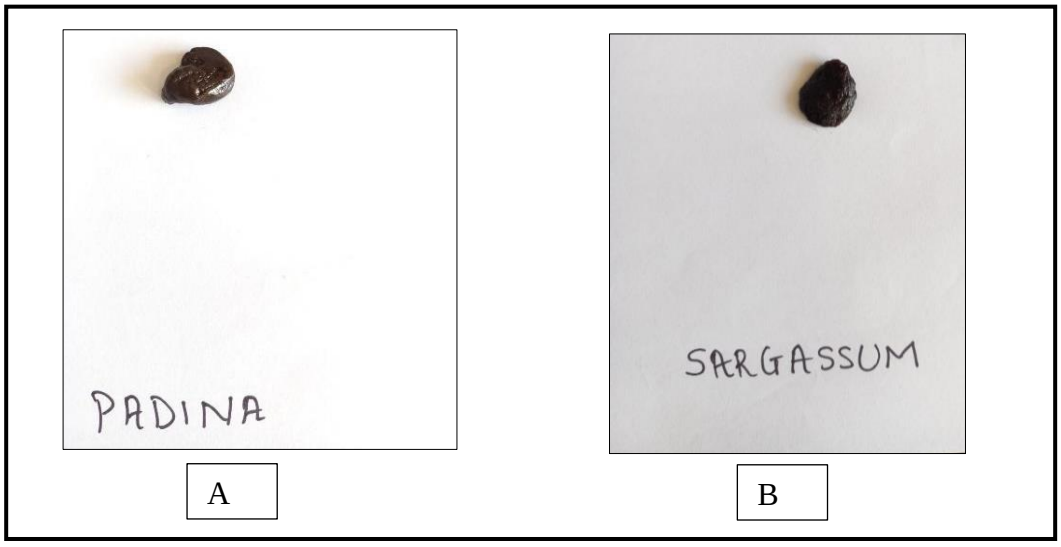

Figure 7: (A) Polysaccharide extracted from *Padina* sp. (B) Polysaccharide extracted from *Sargassum* sp.

5.3.2 Estimation of Moisture Content

The moisture content was estimated using the given formula and recorded as seen in Table 5.2

Table 5.2: Moisture Content Estimation.

Sample	**Moisture content (%)**
Padina alginate	72.19
Sargassum alginate	45.89

According to a study by Rashedy et al.,(2021), *Padina* sp. and *Sargassum species* showed a moisture content of 13.1 % and 12.5 % respectively. In another study by Mokoginta et al., (2019) *Sargassum* sp. showed a moisture content of 9.61%.

The texture, flavour, and appearance of the ingredients may change depending on the amount of water they contain. The presence of water content in dry materials can lead to damage because of chemical reactions and microbiological growth.

The polysaccharide extracted in this experiment showed moisture content much greater than those seen in other studies. The Food Chemical Codex (FCC) states that the moisture content in sodium alginate should be < 15% water. Therefore alternative technique of drying would need to be applied in order to reduce the moisture content to produce alginate of FCC standard.

5.3.3 Estimation of Viscosity

Table 5.3: Reduced Viscosity Estimation.

Sample	**Viscosity (mL/g)**
Padina alginate	18.2
Sargassum alginate	18.2

In the study by Rashedy, (2021) the viscosity of alginate extracted from *Padina* was seen to be 57.6 cP and from *Sargassum* was 128.4 cP. According to FCC standard the viscosity of sodium alginate ranges between 10 to 5000 cP. However, a number of variables, including the ratio of M and G units in the extracted alginate, temperature, and acidic media, have an impact on the viscosity of the alginate.

5.3.4 Evaluation of Purity of Alginate Polysaccharide by Qualitative Phytochemical Analysis

5.3.4.1 Test for Flavonoids

No yellow coloration of the solution was observed for either of the crude polysaccharide samples. The solution did not turn colourless. Therefore, Flavonoids are suggested to be absent in the crude polysaccharide samples extracted from *Padina* sp. and *Sargassum* sp.

5.3.4.2 Test for Alkaloids

No creamy white precipitate was observed for either of the crude polysaccharide sample solutions. Therefore, Alkaloids were found to be absent in both the crude polysaccharide samples extracted from *Padina* sp. and *Sargassum* sp.

5.3.4.3 Test for Tannins

No blue- black or brownish-green color was observed in either of the crude polysaccharide sample solutions. Therefore, tannins were found to be absent in both crude polysaccharide samples extracted from *Padina* sp. and *Sargassum* sp.

It was observed that all the qualitative phytochemical test showed negative results indicating the absence of other seaweed metabolites such as tannins, flavonoids etc. A similar result was seen in a study by Rashedy et al., (2021).

5.3.5 Biochemical Analysis of Alginate Polysaccharide

5.3.5.1 Carbohydrates Analysis

Padina sp. showed a higher concentration of carbohydrates (9%) than *Sargassum* sp. (8%). Marine algae are rich sources of carbohydrates. Carrageenan, fucoidin and alginates for example are most widely used carbohydrates in the food industries. According to a study by Rashedy et al.,(2021), *Padina* sp. and *Sargassum* species showed 76.3% and 83.1% of carbohydrates respectively. Our

extracted polysaccharide shows significanlty lower amount of carbohydrates on comparison with the results seen in Rashedy et al.

5.3.5.2 Total Protein Analysis

Padina sp. showed a much higher concentration of protein (1.43%) than *Sargassum sp.* (0.44%). Peptides, enzymes, glycoproteins, lectins, mycosporine-like amino acids, and phycobiliproteins are present in marine algae. According to a study by Rashedy et al.,(2021), *Padina sp*. and *Sargassum species* showed 0.86% and 0.95 % of proteins which is comparatively much higher than our extracted polysaccharide.

5.3.6 Antioxidant Analysis for Alginate Polysaccharide

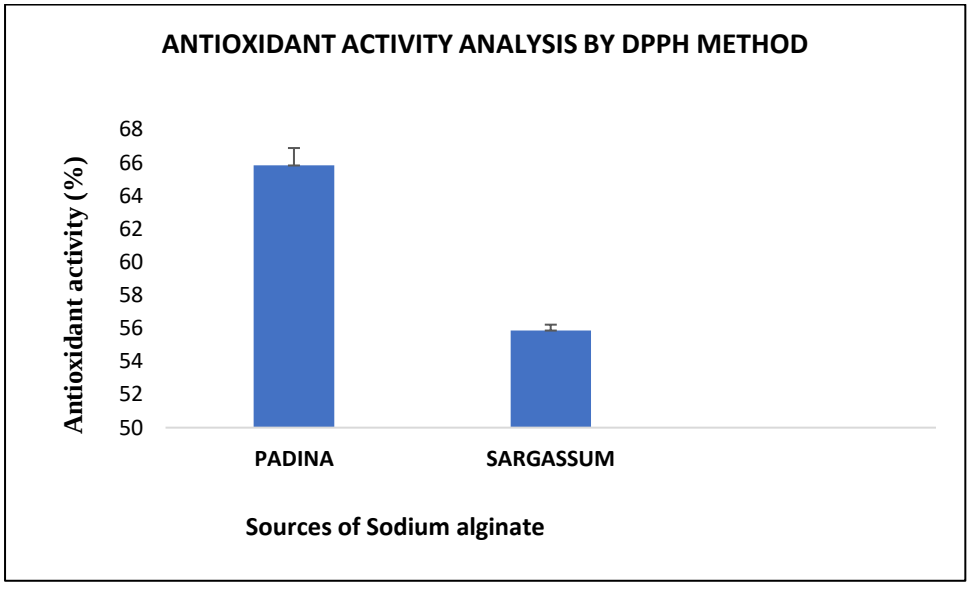

Figure 8: Graph of antioxidant activity in alginate extracted from *Padina sp*. and *Sargassum sp.*

Padina sp. showed a much higher antioxidant activity (65.8 %) than *Sargassum* sp. (55.8 %). The highest absorbance of the stable free radical DPPH is observed at 517 nm. The radical is scavenged and the absorbance would decrease when DPPH comes into contact with a proton-donating molecule like an antioxidant (Wu, Chen, & Shiau, 2003).

5.4 Synthesis of Biopolymer Film

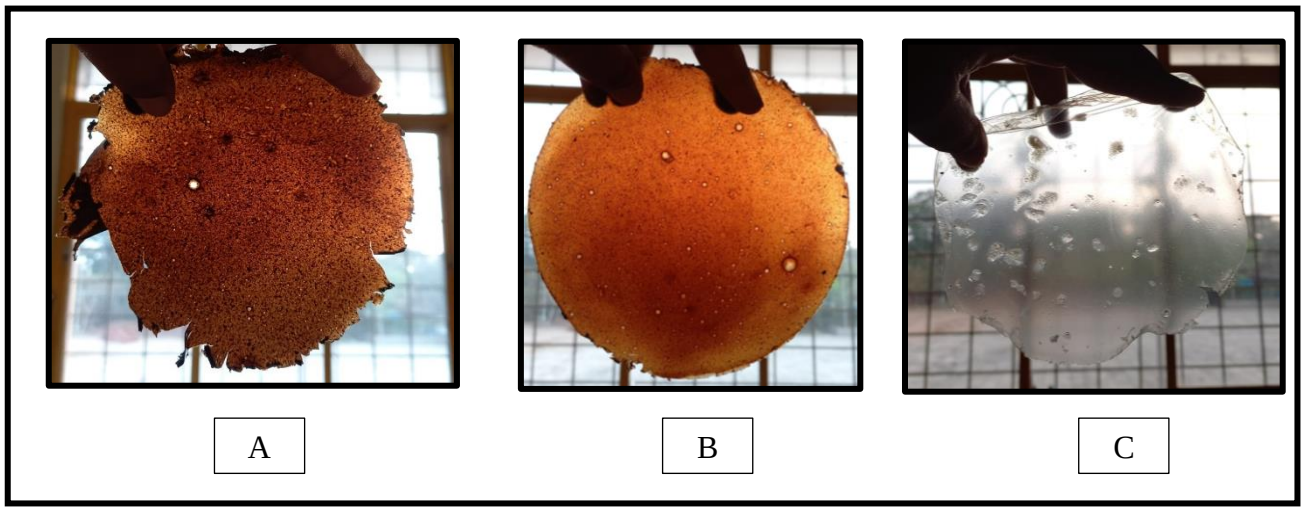

Figure 9: (A) *Padina* biofilm. (B) *Sargassum* biofilm. (C) Standard alginate biofilm

5.5 Characterization of Biopolymer Film

5.5.3 Physical Properties

5.5.3.1 Thickness

The thickness was measured and recorded as seen in Table 5.4

Table 5.4: Thickness Estimation

Sample	Thickness (mm)
Padina biofilm	0.22 ± 0.16
Sargassum polysaccharide biofilm	0.25 ± 0.18
Standard alginate biofilm	0.10 ± 0.08

According to a study by Norajit et al., (2010), bioplastic film made of alginate and glycerol showed a thickness of 0.070 mm. In a study by Rhim (2004), they showed that biofilm made of alginate and glycerin had a thickness of 0.06 mm. The safety of food goods depends greatly on the thickness of the packing films. As layer thickness grows, it may become more pest resistant and gas permeability (Guidara et al., 2020). While the biofilm prepared in this experiment with the extracted polysaccharide was thicker in comparison to the one reported by Norajit et al. and Rhim (2004), it could imply better barrier benefits to the food being packaged.

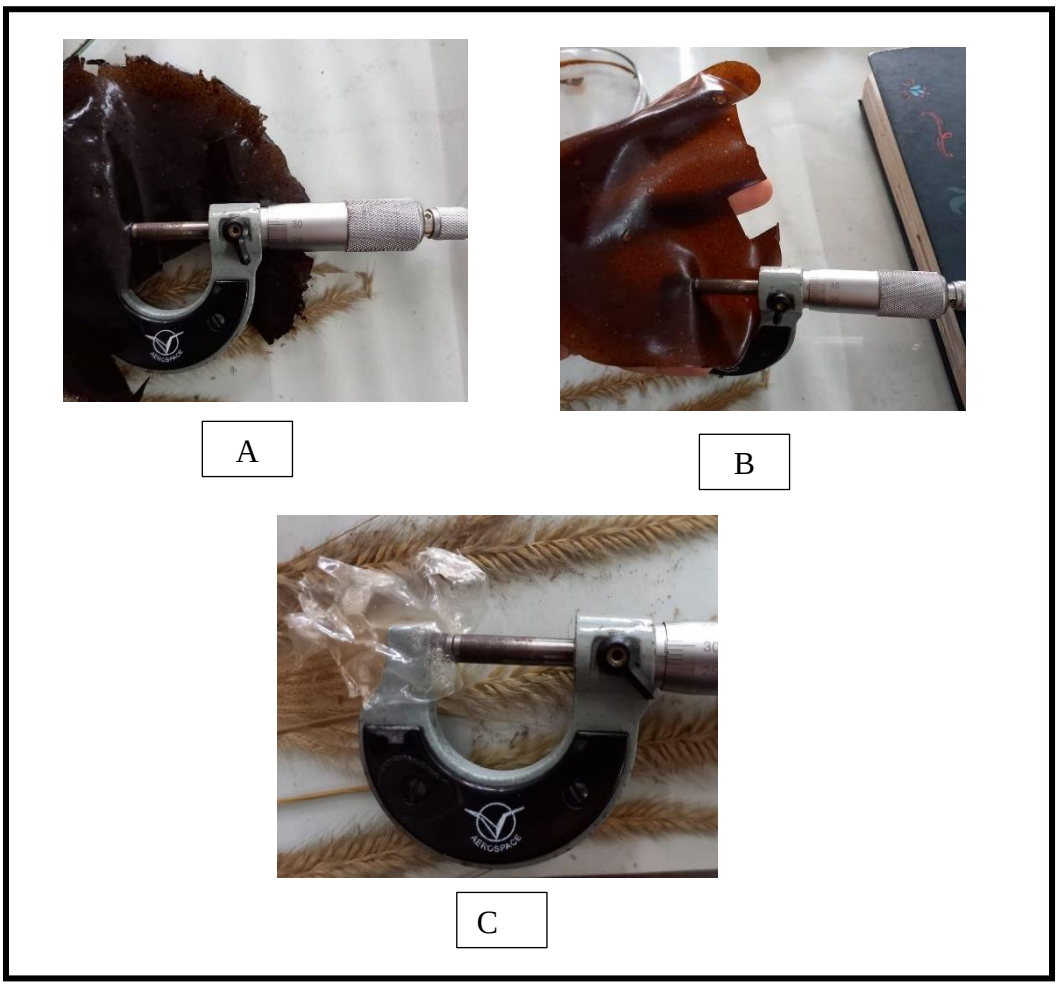

Figure 10: (A) Measurement of thickness of *Padina* biofilm with Screw gauge Micrometer. (B) Measurement of thickness of *Sargassum* biofilm with Screw gauge Micrometer. (C) Measurement of thickness of Standard biofilm with Screw gauge Micrometer.

5.4.1.2 Solubility

The solubility was calculated using the given formula and recorded as seen in Table 5.5

Table 5.5: Solubility Estimation

Sample	**Solubility (%)**
Padina polysaccharide biofilm	78.66 ± 10.77
Sargassum polysaccharide biofilm	69.05 ± 16.20
Standard alginate biofilm	87.5 ± 12.5

Water solubility may have an impact on the usage of biofilm as a packaging material. While some food products require water-soluble biofilm packaging before consumption, others require water-insoluble bioplastic to preserve their qualities and extend shelf life. However, the majority of food packaging uses biofilms to prevent food spoiling that could result from the moisture impact; as a result, biofilm resistance to moisture sorption is greatly desired (Oluwasina & Awonyemi, 2021). In a study by Norajit, (2010), they reported that biofilm samples made of alginate and glycerol showed 77.77% water solubility. The polysaccharide biofilm from *Padina* sp. showed similar results with 78.66 % whereas the polysaccharide film from *Sargassum* and the standard alginate biofilm showed results in a range of 60 to 85 % water solubility.

5.4.1.3 Moisture content

The moisture content was calculated using the given formula and recorded as seen in Table 5.6.

Table 5.6: Moisture Content Estimation

Sample	Moisture Content (%)
Padina polysaccharide biofilm	27.60 ± 4.24
Sargassum polysaccharide biofilm	19.71 ± 7.23
Standard alginate biofilm	32.64 ± 1.20

A food product's shelf life may be affected by the amount of moisture it contains; a high moisture content would accelerate decay because it would promote microbial development. Therefore, lower moisture content would help prevent the bioplastic's early deterioration (Oluwasina & Awonyemi, 2021).

Norajit, (2010) in a study reported that biofilm made up of alginate and glycerol showed a moisture content of 29.67 % which was in the range in which the test samples of this experiment fall i.e. 20 - 33%. Being extremely similar to the *Padina* polysaccharide biofilm.

5.4.1.4 Transparency

The Transparency was calculated using the given formula and recorded as seen in Table 5.7

Table 5.7: Transparency Estimation

Sample	Transparency
Padina polysaccharide biofilm	7.42 ±0.23
Sargassum polysaccharide biofilm	5.69 ±0.12
Standard alginate biofilm	1.75 ±0.12

The consumer acceptance of a packaged food may be impacted by transparency. High transparency (low value) is desirable when it comes to transparency because it affects the packaging aesthetic (Lim et al.,2021). In a study by Norajit, (2010), biofilm made up of alginate and glycerol showed 0.88 % transmittance which was lower when compared to the test samples in this experiment.

5.4.2 Morphological Properties

5.4.3.1 SEM- EDX

The scanning electronic microscopy images were studied focusing on the morphology of the biofilms. It was seen that the *Padina* sample and the *Sargassum* sample were rougher compared to the standard alginate sample. The standard alginate biofilm showed more visible cracks whereas the extracted polysaccharide samples showed fewer cracks.

EDX analysis was carried out and the element composition of the biofilms was found. Carbon, Oxygen and Sodium were the main elements reported in all three biofilms whereas *Padina* biofilm and *Sargassum* biofilm also showed presence of Chlorine and Potassium.

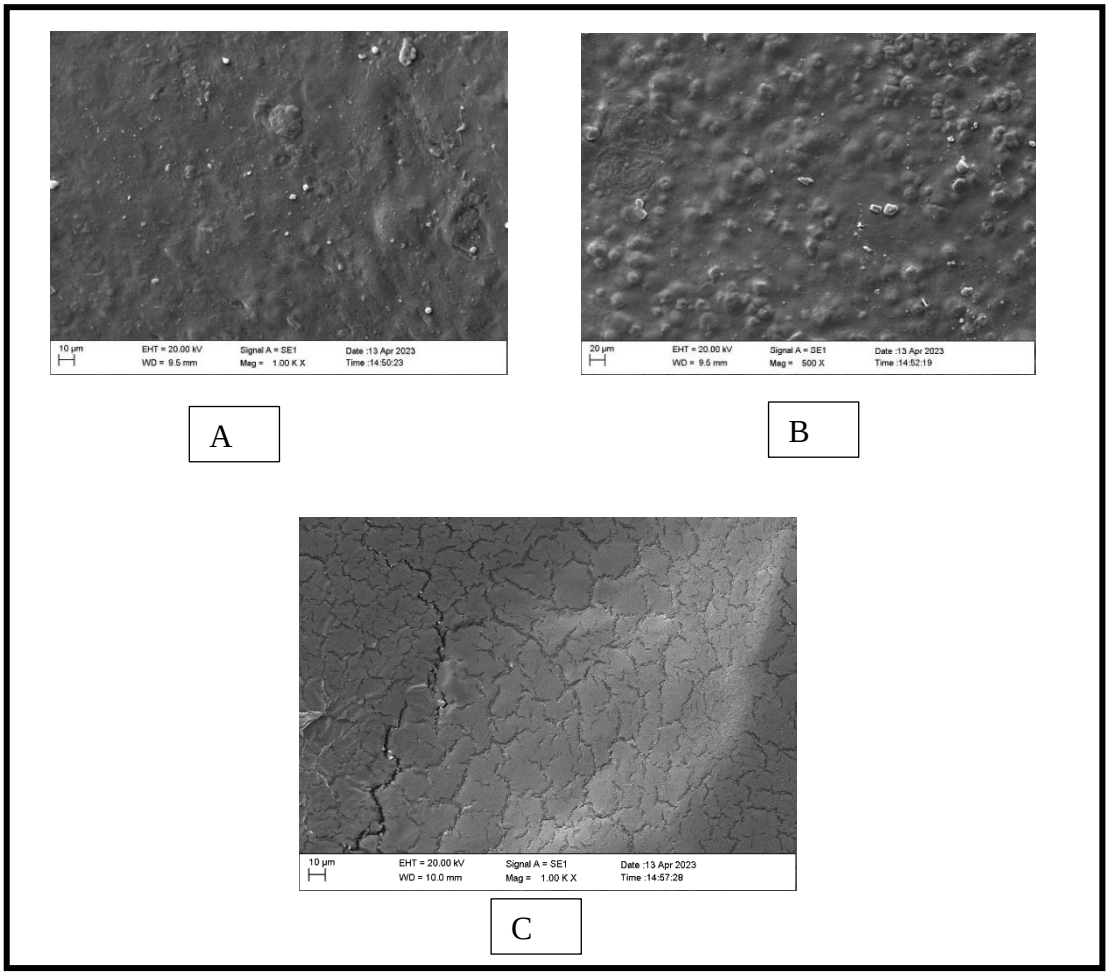

Figure 11 : (A) SEM analysis of *Padina* alginate biofilm. (B) SEM analysis of *Sargassum* alginate biofilm (C) SEM analysis of standard alginate biofilm

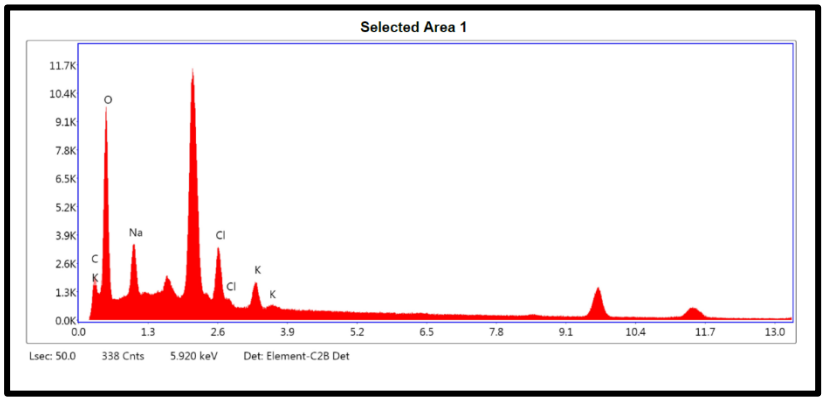

Figure 12: EDX analysis for *Padina* alginate biofilm.

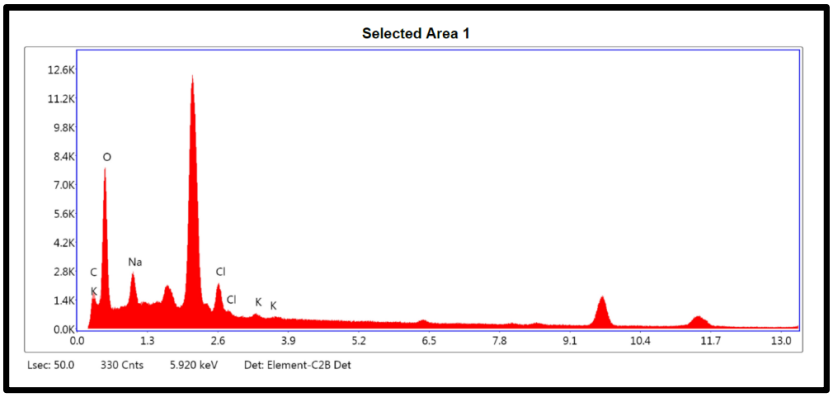

Figure 13: EDX analysis for *Sargassum* alginate biofilm.

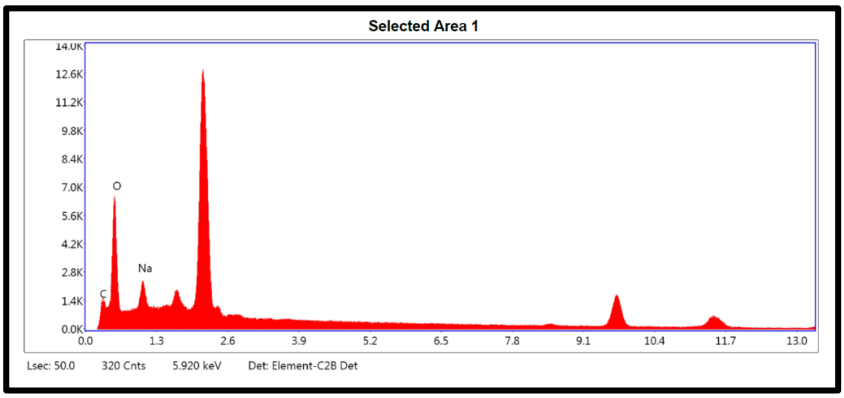

Figure 14: EDX analysis for Standard alginate biofilm.

5.4.4 Anti-Oxidant Properties

5.4.4.1 DPPH

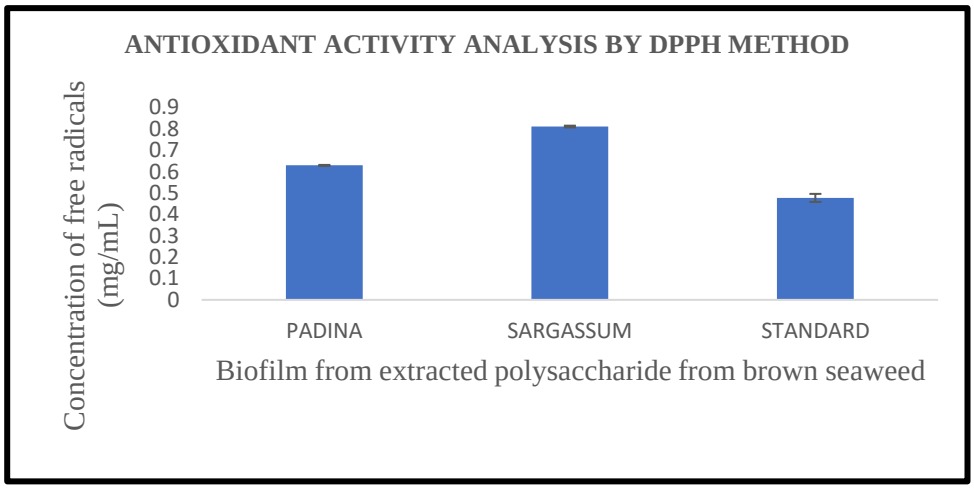

Figure 15: Graph of antioxidant activity in biofilm from alginate extracted from *Padina* sp. and *Sargassam* sp.

Sargassum sp. bioplastic showed a much higher antioxidant activity (0.810mg/ml) than *Padina sp. bioplastic* (0.628mg/ml). Bioplastic made from standard lab grade alginate showed the lowest antioxidant activity (0.476mg/ml)

5.5 Antioxidant properties of biofilm

It was observed that the pieces of apple within the biofilm took longer to oxidise than those kept exposed to atmospheric air.

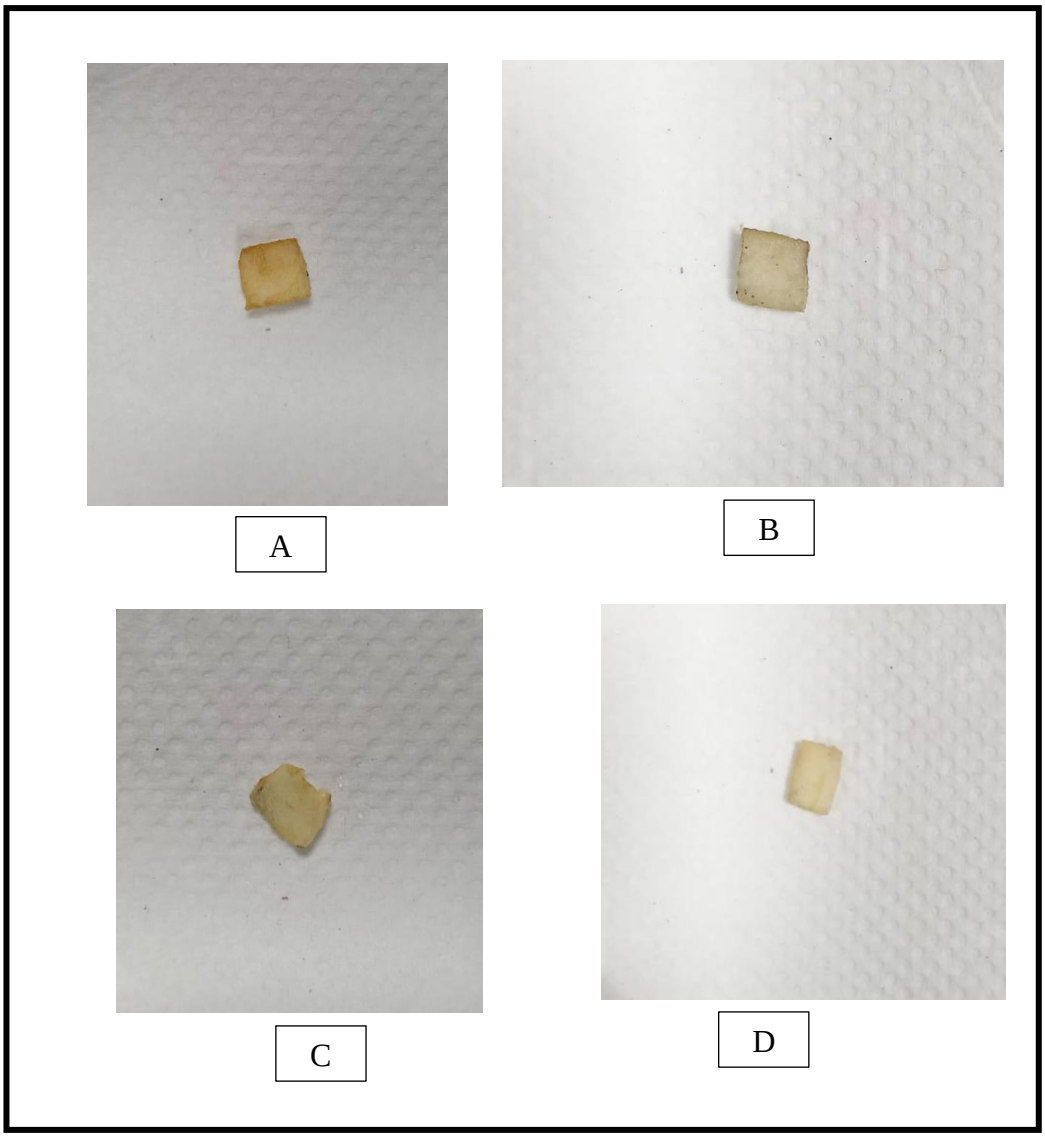

Figure 16 : (A) Apple piece kept exposed to air (B) Apple piece after removing from *Padina* biofilm packet (C)Apple piece kept exposed to air (D) Apple piece after removing from *Sargassum* biofilm packet.

SUMMARY AND CONCLUSION

Summary

Brown seaweeds namely *Padina tetrastromatica*, and *Sargassum cinereum* were collected from Odxel and Vainguinim beach of Goa's coastline.

A polysaccharide sodium alginate was extracted from both seaweeds using hot alkali extraction method. *Padina* sp. showed a yield of 23.6% and *Sargassum* sp. showed a yield of 38.64% and was the highest yield.

The crude polysaccharides extracted from both seaweeds was characterized and was found to be devoid of flavonoids, alkaloids and tannins. The polysaccharide showed a brown color and pH of 9.98 in *Padina* sp. whereas *Sargassum* sp. showed a lower pH of 4.77. *Padina* sp. also showed higher moisture content of 72.19% and *Sargassum* sp. showed moisture content to be 45.89%.

Padina sp. were also seen to have a higher concentration of carbohydrates (9%) and proteins (0.143 mg/ml) and showed higher antioxidant activity (0.658mg/ml) than *Sargassum sp.* which showed lower carbohydrate (8%), protein (0.044 mg/ml) and antioxidant activity (0.558mg/ml).

The extracted crude sodium alginate polysaccharides were used to develop biopolymer films. The Standard alginate biopolymer film showed best bioplastic characteristics with 0.144 mm thickness, 87.5 % solubility and 2.432 transparency. Sargassum showed better bioplastic characteristics in terms of antioxidant activity (0.810 mg/ml) and moisture content (19.705 %)

Conclusion

The study provides data for bioprospecting the use of brown seaweeds from the coast of Goa for its use in the food industry.

Out of the two seaweeds that were isolated, *Sargassum* sp. showed highest yield of sodium alginate polysaccharide as compared to *Padina* sp.

Padina sp. extracted polysaccharide showed better characteristics of pH, moisture content, antioxidant activity and carbohydrate composition compared to the polysaccharide extracted from *Sargassum* sp.

Biopolymer films prepared using standard lab grade sodium alginate showed best bioplastic characteristics, followed by *Sargassum* extracted polysaccharide biopolymer film. Further characterization of the biopolymer and standardization of the production process could aid in developing biodegradable food packaging material from seaweeds for the food industry.

FUTURE PROSPECTS

- Isolation and extraction of polysaccharides such as ulvan from green seaweed, carrageenan from red seaweed and fucoidan from brown seaweed could be carried out to test their potential in the food industry.
- Further characterization of crude sodium alginate by FTIR analysis could be performed.
- Improvement in the film's characteristics and further characterization of biopolymer films to detect antimicrobial potential, toxicity levels, thermal properties, biodegradability could be performed.
- Application of these biopolymer film for edible packaging and smart packaging could be performed.

APPENDIX

1. **Meyers reagent:**

   Mix 1.3 g of mercuric chloride and 5 g of potassium iodide in 100 mL of distilled water.

2. **DNSA:**

   Mix 3 g of sodium potassium tartrate in 50 mL of distilled water.

   Mix 1 g of DNSA powder in 20 mL of 2 M NaOH.

   Mix both solutions together.

3. **Reagent C**

   Reagent A: Dissolve 0.1 g of NaOH in 100 mL of distilled water.

   Add 2 g of Na_2CO_3 to the above solution and dissolve.

   Reagent B: Dissolve 1 g of Na-K-T in 100 mL of distilled water.

   Add 0.1 g of $CuSO_4$ to it and dissolve.

   Mix 50 mL 0f Reagent A with 1mL of Reagent B to get Reagent C.

4. **DPPH**

   Dissolve 11.82 g in 100 mL of 70 % Ethanol.

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