# Bioplastic from *Gracilaria corticata* (J. Agardh) J. Agardh polysaccharides with nanosilica reinforcement

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**By**

**JENNATH SHERIN A**

**Reg No: AM20BOT012**


**DEPARTMENT OF BOTANY AND CENTRE FOR RESEARCH**

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# CERTIFICATE

This is to certify that the dissertation entitled **"Bioplastic from *Gracilaria corticata* (J. Agardh) J. Agardh polysaccharides with nanosilica reinforcement"** is an authentic record of work carried out by **Jennath Sherin A**, under my supervision and guidance in the partial fulfilment of the requirement of the M. Sc. Degree of Mahatma Gandhi University, Kottayam. I, further certify that no part of the work embodied in this dissertation work has been submitted for the award of any other degree or diploma.

Anu Joy

Supervising Teacher

Department of Botany and centre for research

St. Teresa's College (Autonomous)

Ernakulam

Dr. Liza Jacob

Head of the Department of Botany and centre for research

St. Teresa's College (Autonomous)

Ernakulam

Place: Ernakulam

Date: 25.5.2022

External Examiners

1. K. Maathusudhanan 13/6/22

2. Dr. Stephy Sqheira 13/06/2022.

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**Place: Ernakulam** **JENNATH SHERIN A**

**Date:**

# CONTENT

# INTRODUCTION

Nowadays, most packaging materials are still derived from non-renewable sources. These synthetic polymers have been used as packaging materials for many years due to their economic and technological advantages, such as high availability, low cost, and favorable functional properties. However, such polymers present hydrophobic nature, which limits the action of microorganisms, and therefore takes many years to decompose. This results in the production of large volumes of solid wastes, which leads to serious environmental problems (Tharanathan, 2003). A total of 1.3 billion tonnes of municipal solid waste per year was generated in 2012, but it is expected to increase to 2.2 billion tonnes per year by 2025. Non-renewable, non-biodegradable packaging materials have serious environmental drawbacks. They have been considered a major source to the solid waste and environmental pollution by consumers and environment activists (Risch, 2000; Ramos *et al*., 2013). These issues have been greatly aggravated due to the increase in population and economic growth from developed and developing countries. Hence, the need to reduce the amount of discarded plastics is being recognized globally, aiming to replace them with packaging films based on biodegradable materials, which are recognized as environmentally friendly materials (Tharanathan, 2003).

In order to solve this problem, companies and researchers have been working on ways to develop new packaging strategies with environmentally friendly, abundant biodegradable packaging materials made from renewable natural polymers (Risch, 2000; Gontard and Guilbert, 1994). Furthermore, the rapidly growing interest in the use of edible packaging can also be associated with a growing interest from consumers for minimally processed fresh-like foods with an extended shelf life and trend in improving the quality of food with edible barriers (Diab *et al*., 2001).

Packaging plays a very important role in food preservation and in health-enhancing foods (Cerqueira *et al*., 2010). Food packages usually act as inert barriers for product protection with no interaction with food. However, edible films can provide additional protection for food, while being a fully biodegradable and environmentally friendly packaging system. Biofilms, as the primary barrier against physical impacts, prevent contamination, increase shelf life and contain important information about packaged food. In addition, biofilms can be used to

improve food quality, as they can carry functional ingredients such as fatty acids, antioxidants, antimicrobials, nutrients, and flavors to further enhance food quality, stability, functionality, and safety (Lin and Zhao, 2007).

Edible films and coatings are thin layers of material (their thickness is generally less than 0.3 mm) used for enrobing the food product to replace or fortify the natural layers and can be consumed as a part of the product or with further removal (Guilbert and Gonard, 1995; Pavlath and Orts, 2009). Therefore, the materials used in the formulation should conform to the general food laws and regulations (Guilbert *et al*., 1996). Additionally, the coatings and films should not affect the organoleptic properties of the food product negatively (Gontard and Guilbert, 1994).

Edible packaging can be a superficial coating on the food or continuous layers between compartments/ingredients of the heterogeneous products (e.g., pizza, bakery fillings, and toppings) (Guilbert and Gonard, 1995). The coating can also be applied on individual pieces of the whole product, which have not been individually packaged due to practical arguments, such as fresh-cut melons, kiwis, strawberries, nuts, beans, pears (Bourtoom, 2008).

Edible films and coatings can be used to overcome many obstacles involved in the marketing of foods (Donhowe and Fennema, 1994). These functions can be specified as retarding moisture, gas, solute and oil migration, improving structural integrity, retaining volatile flavor compounds, conveying food additives (Donhowe and Fennema, 1994). In addition, they improved the aesthetic appearance by minimizing the development of physical damage, hiding scars, and improving surface shine (Ncama *et al*., 2018; Murmu and Mishra, 2018). For instance, hot-melt paraffin waxes have been used to coat citrus fruits to retard moisture, edible collagen casings have been used for sausages to provide structural integrity and apples have been coated with wax to improve surface shine and prevent physical damage.

The required features expected from edible films and coatings can be assigned by the specific characteristics of the product and changes during production, transportation, and storage periods. Despite providing a barrier, the non-edible packaging is still essential for edible coated food products due to hygienic reasons (Guilbert and Gonard, 1995). Nevertheless, combining edible films and coatings with traditional packaging would likely reduce the non-biodegradable packaging waste of processed foods and environmental effluence (Donhowe and Fennema, 1994; Shit and Shah, 2014; Dehghani *et* al., 2018).

The most common materials used in the formulation of edible films are proteins (e.g., gelatin, casein, wheat gluten, and zein) and polysaccharides (e.g., alginate, starch, and chitosan), which are used alone or blended. These biopolymers are highly biodegradable and decompose easily into inorganic by-products like carbon dioxide and water (Santacruz *et al*., 2015).

Seaweeds are important autotrophic and photosynthetic plants, which are now often explored frequently due to the presence of some vital polysaccharides such as alginate, agar, floridean starch and carrageenan (Rajendran *et al*., 2012).

Polysaccharides are widely distributed in nature as they can be derived from plants, animals and microorganisms. Furthermore, variation in physicochemical properties, such as mechanical properties, solubility, viscosity, gelling potential, surface and interfacial properties, governed by monosaccharide composition, chain length (degree of polymerization), linkage types and patterns, provide polysaccharides versatility in preparation of materials with diverse applications. In fact, polysaccharides based materials in different forms including fibers, films, food casing, membranes, hydrogels, aerogels and sponges, with applications in several important commercial areas such as food, pharmaceuticals, biomedical, electronics, and adsorption have been developed (Hu and Abidi, 2016; Hu and Catchmark, 2011; Hu *et al*., 2013; Hu *et al*., 2014; Hu *et al*., 2016; Hu *et al*., 2016; Wang *et al*., 2016).

These carbon containing polymers are found to be potentially active in the formation of bio-plastics (Rajendran *et al*., 2012; Wang and Rhim, 2015; Farhan and Hani, 2017). Similarly, agar, that is present intracellularly within the cell walls of red seaweed (Martins *et al*., 2012; Gade *et al*., 2013; Wang and Rhim, 2015; Tabassum, 2016; Hii *et al*., 2016) possess good gelling & emulsifying abilities and is reviewed in several studies as a promising agent for bioplastic synthesis (Wu *et al*., 2009; Hii *et al*., 2016; Tabassum, 2016). Bioplastics prepared from agar are shown to have fine physical and mechanical properties besides good flexibility and tensile strength enabling their feasible use in commercial utilization (Arham *et al*., 2016). These plastics are also known to solubilize and decompose easily preventing their long residence time on the lands and as well as in waters (Hii *et al*., 2016; Arham *et al*., 2016; Hira *et al*., 2018). In the preparation of such eco-friendly plastics, plasticizers play a significant role by improving the elasticity and ductility of plastics (Vieira *et al*., 2011). These non-volatile compounds with low molecular masses, accumulate within the polymer chains and reduce their intramolecular forces thus providing the resistance against breaking or deformation (Bourtoom, 2008; Mali *et al*., 2008; Vieira *et al*., 2011; Felix *et al*., 2016)

The application of nanostructured materials in packaging materials can improve the existing qualities of food packaging materials (Enescu *et al*., 2019). Nanostructured materials are those in which the structural constituents have at least one dimension in nanometric scale and the diameters ranging from 1 to 100 nm. Nanostructured materials include nanoparticles, nanorods, nanowires, thin films, and bulk materials made from nanoscale building blocks or containing nanoscale features. Nanostructured materials are categorized as zero-dimensional (nanoclusters, quantum dots, fullerenes), one-dimensional (nanorods, nanotubes), two-dimensional (ultrafine-grained over layers, thin films), or three-dimensional (particles, nanocomposites, dendrimers) nanomaterials. Nanotechnology has paved the way for the use and development of novel nanostructured materials known as nanomaterials in the food packaging sector (Sharma *et al*., 2017)

Nanomaterials provide a range of functional characteristics in the packaging, such as improving thermal, barrier, and mechanical properties. Therefore, there is a growing need for nanomaterials in food system packaging. Nanomaterials are used in food items to modulate the release of *antioxidants*, flavors, enzymes, and antimicrobials (Huang *et al*., 2018). Nanomaterials included in food packaging materials release active ingredients in a regulated manner and prevent food spoiling (Mishra *et al*., 2018). As a result, nanomaterials in food packaging enhance the usage of biodegradable materials in packaging, reduce wastage of processed foods, preserve food freshness, and increase shelf life (Tosif *et al*., 2021; Chawla *et al*., 2021).

Polysaccharide-based nanoparticles can be used as fillers to improve the physical properties of biopolymers. The application of bio-nano composites in industrial packaging is being researched as part of the continuous quest for novel solutions for efficient and sustainable systems. Bio-nanocomposites are eco-friendly components made from biodegradable and renewable material and hence referred to as green nanocomposites. Bio-nano composites have antibacterial characteristics that allow them to inactivate bacteria more efficiently due to the increased surface-to-volume ratio and higher surface reactivity of the nanosized antimicrobial agents (Altaf *et al*., 2022).

Advances in food packaging researches led to the development of active packaging, and intelligent packaging. Active packaging is a novel method used to prolong the shelf-life of perishable foods, maintain or improve the quality and safety of prepared foods due to its interaction with the product. Besides, active packaging has potential to replace the addition of

active compounds into foods, reduce the movement of particles from packaging materials to food, and get rid of industrial processes that can cause the introduction of pathogenic microorganism into the product (Schaefer and Cheung, 2018). In addition, bioactive packaging contains antimicrobial agents that interact with biological molecules and may inhibit the growth of various microorganisms (Brockgreitens and Abbas, 2016)

Using oxygen scavenging or absorbing agents like Ascorbic acid in edible biofilms, offers several benefits, such as inhibiting the formation of microbial growth, maintaining the quality of lipid-containing foods (preventing rancidity), avoiding discoloration, and avoiding oxidation.

The ascorbic acid (C6H8O6) – better known as vitamin C – is an organic compound belonging to the family of monosaccharide. It is strongly water-soluble and it is often considered as one of the elements that characterises the Mediterranean diet (Ferro-Luzzi *et al*., 1994). The diffusion of its use is also most important in the food industry, which has always used its stabilised and antioxidant property. Indeed, there are several formulations of additives that contain ascorbic acid (Liao and Seib, 1988).

One of the most important characteristics of the ascorbic acid is its reducing ability. In the presence of oxygen, ascorbic acid tends to oxidise with a strong result, especially in relation to catalyst metals, removing the environmental resources of oxygen. Furthermore, the ascorbic acid can react with free radicals, arresting the chain reactions that may provoke dangerous effects on microorganisms (Cerutti, 2006). It allows maintaining stable other important elements, such as vitamin A, E, folic acid and thiamine in organisms and foods (Mora – Gutierrez and Gurin, 2006).

Oxygen scavengers are widely used in this food industry, as they extend the shelf life of products from 3–4 to 14 days or more (Gaikwad and Lee, 2016; Singh *et al*., 2016a; Singh *et al*., 2016b; Singh *et al*., 2016c). Currently, commercial oxygen scavengers take many forms, including sachets, films (directly in the package), and labels. However, incorporating scavengers directly into packaging materials has better consumer acceptance than using sachets. The oxygen scavengers market is expected to reach USD 2.41 Billion by 2022, at a compound annual growth rate (CAGR) of 5.1% from 2017 to 2022. Mitsubishi Gas Chemical Company (Japan), BASF SE (Germany), Ecolab Inc. (USA), Clariant Ltd. (Switzerland), and Kemira OYJ (Finland) are the leading players operating in the oxygen scavengers market (Anonymous, 2017a; Anonymous, 2017b). The increasing demand for high-quality packaged

food is one the major drivers for the growth of oxygen scavengers market. Factors such as increasing disposable income and changing lifestyle of the middle-class population in emerging countries are expected to fuel the demand for packaged food. This increase in demand is consequently expected to drive the growth of the oxygen scavengers market in the coming years. The growing awareness regarding the reduction of food wastage and the increased demand for advanced packaging among consumers are expected to further aid the growth of the market during the forecast period. Oxygen scavenging films extend the shelf life of foods while maintaining their nutritional quality and preventing discoloration, microbial spoilage, rancidity, and organoleptic deterioration, thereby ensuring food safety (Cichello, 2015).

It has also been reported that, direct addition of aromatic plant essential oils and extracts to foodstuffs can also exert an antioxidant or antimicrobial effect (Costa *et al*., 2015). Among compounds of natural origin, biological activities have been shown by essential oils from aromatic and medicinal plants and have received particular attention because of their radical-scavenging properties (De Sousa Barros *et al*., 2015).

Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Plant extracts and essential oils possess antifungal, antibacterial, and antiviral properties and have been screened on a global scale as potential sources of novel antimicrobial compounds, agents promoting food preservation, and alternatives to treat infectious diseases (Safaei-Ghomi and Ahd, 2010; Astani *et al*., 2010). Essential oils have been reported to possess significant antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal, and insecticidal activities (Kaloustian *et al*., 2008; Benijilali and Ayadi, 1986; Burt, 2004). Therefore, essential oils can serve as a powerful tool to reduce the bacterial resistance (Stefanakis *et al*., 2013). Aromatic oily liquids called essential oils (also called volatile oils) are obtained from plant materials (leaves, buds, fruits, flowers, herbs, twigs, bark, wood, roots and seeds). An important characteristic of essential oils and their components is hydrophobicity, which enables them to partition with the lipids present in the cell membrane of bacteria and mitochondria, rendering them more permeable by disturbing the cell structures. This eventually results in the death of bacterial cell due to leakage of critical molecules and ions from the bacterial cell to a great extent (Devi *et al*., 2010).

The present study is conducted to;

- Extraction of polysaccharide from a seaweed, *Gracilaria corticata*
- Using sol-gel method, extraction of nanosilica particles from rice husk

- Preparation of biodegradable edible film from polysaccharide and incorporation of nanosilica particles
- Addition of a plasticizing agent, glycerol, to reduce the brittleness of the film
- Integration of a potential antioxidant agent; Ascorbic acid, into the biopolymeric film
- Estimation of various characteristic properties of the polysaccharide, nanosilica particles and the final product; Biofilm, using different analytic tests.

# REVIEW OF LITERATURE

In recent years, there has been an increasing consumer demand for fresher and healthier foods in global markets (Espitia *et al*., 2014; Mostafavi *et al*., 2017). This has increased the use of plastic-based packaging materials for maintaining the quality of products and increasing their shelf-lives (Mahalik and Nambiar, 2010). Increasing concerns over environmental pollutions made by plastics led to the development of biodegradable packaging films (Tavassoli-Kafrani *et al*., 2016; Sedayu *et al*., 2019). Edible films could be suitable alternatives for plastics in various applications due to their abilities in preventing the transfer of moisture, oxygen, and aromas between food and its surrounding atmosphere (Cazón *et al*., 2017)

According to the study conducted by Mostafavi and Zaeim, 2020, on Agar-based edible films for food packaging applications, they have concluded that, Agar, as a non-toxic biodegradable biocompatible polysaccharide, can form continuous and transparent films with heat sealability. However, in comparison with plastic-based packaging materials, pure agar film is relatively brittle and has low elasticity, poor thermal stability, medium gas barrier properties, high water sensitivity, and high WVP, which limit its industrial applications.

In a study conducted by Jong-Whan Rhim *et al*., 2011, the prepared agar matrix was treated with three different types of nanoclay (Cloisite $Na^+$, Cloisite 20A and Cloisite 30B) to incorporate nanosized particles into the film. The dispersion property was then compared in all the three cases. As indicated by Sothornvit, et al., 2009, the type of nanoclays greatly influenced the degree of dispersion in the film-forming solution. Among the clays tested, the natural MMT (Cloisite $Na^+$), which is hydrophilic, was dispersed best in the film-forming solution followed by less hydrophobic Cloisite 30B by a simple mixing with a magnetic stirrer; however, the hydrophobic Cloisite 20A was hardly dispersed in the film-forming solution without further treatment such as high shear mixing homogenization and ultrasonication. Apparently, neat agar film and agar/Cloisite $Na^+$ nanocomposite films were transparent, while agar/Cloisite 30B and 20A nanocomposite films were slightly translucent. For a better insight in the homogeneity and in the microstructure of dried films, SEM images and EDS element analysis were carried out. Compared with the neat agar film, all the nanocomposite films include additional elements such as Na, Al, Si, and Mg, obviously that comes from the nanoclays used. This indicates composite was formed between nanoclays and agar polymer matrix. The SEM images of film surface indicate clay particles are relatively well dispersed in

the polymer matrix and they also show that the neat agar film has smoother surface than the other nanocomposite films (Jong-Whan Rhim *et al*., 2011). From SEM images, Jong-Whan Rhim *et al*., 2011, observed that, agar and agar/Cloisite $Na^+$ nanocomposite film exhibited better mechanical properties as compared to those of agar/Cloisite 30B and agar/Cloisite 20A nanocomposite films due to its more compact and homogeneous structure. They then used, X-ray diffraction analysis (XRD), to determine the crystallographic structure of the film. The results thus indicated that the degree of intercalation was higher in Cloisite $Na^+$ nanocomposite films compared with other types of nanoclay, which clearly indicated that Cloisite $Na^+$ interacted better with agar than the other type of clays.

The SEM analysis of agar/lignin films performed by S. Shankar *et al*.,2015, observed that the lignin particles were well dispersed into agar biopolymer, and also, as expected, the neat agar film was smooth and compact. The neat agar film was transparent, but the color of the films changed from transparent to dark brown with the addition of lignin. The results showed that the lignin concentration significantly influenced the color of agar/lignin biocomposite films. The light transmission characteristic of the agar and agar/lignin biocomposite films was measured using a spectrophotometer at 280 and 660 nm. the light transmittance of agar film decreased significantly after incorporation of lignin and the degree of decrease was strongly dependent on the concentration of lignin. This drastic reduction in transmittance values might be due to the strong UV light absorbing tendency of lignin (Shankar *et al*., 2015) This absorbance of UV light was due to chromophoric groups present in lignin (Chaochanchaikul *et al*., 2012)

The protective function of an edible film or coating is to prevent the transfer of moisture, oxygen, flavour and/or oil content between food and the surrounding medium and/or between different compartments in a heterogeneous food (Phan *et al*., 2005). Sousa *et al*., 2010, studied the film properties and its application to edible coating by extracting biodegradable agar from *Gracilaria Vermiculophylla.* Films of agar extracted from *G. vermiculophylla* using optimum conditions and commercial agar were made using the knife coating technique (Sousa *et al*., 2008). Plasticizer was added to the biofilms with the intent to increase their flexibility and oxygen permeability (Larotonda, 2007). The functional properties (hygroscopicity, mechanical resistance, and permeability to water vapour and oxygen) of the films as well as the potential application of the agar/glycerol solution to fresh fruit and vegetable preservation was tested. Model fruits and vegetables were coated with the biopolymer/plasticizer solution and compared with a control sample in terms of colour, firmness, weight loss and shelf life. Regarding the

coating application tests, results showed that coatings made with *Gracilaria* extracted agar/glycerol solutions were effective in extending cherry tomatoes shelf life in terms of weight loss and firmness although during the second half of the test period this difference tended to vanish. Visual inspection of the fruits revealed that the control fruits lost their gloss whereas the coated fruits kept a light gloss up to the end of the test. This result is supported by the colour parameter L which indicates higher lightness of coated samples throughout the test. The commercial agar/glycerol formulation used wasn´t able to ensure its adherence to the fruit surface (Sousa *et al*., 2010). The overall results obtained showed that agar extracted from *G.vermiculophylla* constitutes a good and cheap alternative to commercial agar regarding the food packaging application and coating.

In 2020, Fatemeh Kalateh Seifari and Hamed Ahari reported that, the advent of active, biodegradable, renewable, and edible materials creates a novel path for the production of an ecofriendly way for storage, transportation, and extending the shelf life in the food industry. The edible films and coatings are biomaterial incorporated with various natural antimicrobial agents. Essential oils of plants as the natural antibacterial, antiviral, and antifungal components are an appropriate source of bioactive material for producing active edible films and coatings. The nanoemulsion of antimicrobial compounds could be used for enhancing antimicrobial compounds' performance in active edible films and coatings. The intrinsic properties of edible films and coatings such as low mechanical properties and high-water vapor permeability could be enhanced with natural nanocrystals as a new class of edible nanofillers.

Elsa Díaz-Montes and Roberto Castro-Muñoz, 2021, examined the effects of using Chitosan as a Primary Biopolymer for Functional Films. They found that, CS-based edible films and coatings have successfully demonstrated their ability to fabricate different concepts of monolayer or composite films. The ability of CS to form edible films is mainly due to its polycationic nature that allows it to work in conjunction with other biopolymers and additives. This synergy causes strong physical interactions between all the components, which reflects the improvement of the physical, mechanical, and permeability properties. In addition, the bioactive potential of CS-edible films is originated by its intrinsic properties such as antimicrobial, antifungal, and antioxidant capacities. These features make CS potentially interesting compared with other biomaterials. The evidence on CS-edible films and coatings shows that they meet the 2019 requirements to be part of the so-called "green" bio-packaging and be an alternative to synthetic packaging. However, this type of technology is highly targeted towards characteristics that must be adequate and specific for the type of food to be

protected, making the area of edible films or coatings have a great potential for study. Finally, since several bioactive compounds extracted from natural sources are providing various benefits to human health, it is likely that CS will continue to be explored as a support for the incorporation of new functional edible films.

Lokender Kumar *et al.*, 2019, have described, a method of preparing biocompatible antimicrobial alginate polymer from aqueous solution of commercial sodium alginate and aqueous extract of Wakame using aminoglycoside antibiotics. The underlying acid-base mechanism involves interactions between negatively charged oxygen due to dissociated sodium ions in alginate and protonated amine in aminoglycosides. Polymerization efficiency seems to loosely correlate with the number of amines and sulfate ions in aminoglycosides. Slow release of aminoglycosides from alginate polymers is evident from the microbial zone of inhibition. Antimicrobial alginate polymers from Wakame, one of the most invasive species in the world that grows in diverse conditions of vast oceans, provides a sustainable and biodegradable alternative for wound dressing with slow release of antibiotics.

In a study conducted by Maziyar Makaremi *et al*., 2019, they have developed active, healable, and safely dissolvable alginate-pectin based biocomposites that have potential applications in food packaging. The morphological study revealed the rough surface of these biocomposite films. Tensile properties indicated that the fabricated samples have mechanical properties in the range of commercially available packaging films while possessing excellent healing efficiency. Biocomposite films exhibited higher hydrophobicity properties compared to neat alginate films. Thermal analysis indicated that crosslinked biocomposite samples possess higher thermal stability in temperatures below 120 ◦C, while antibacterial analysis against E. coli and S. aureus revealed the antibacterial properties of the prepared samples against different bacteria. The fabricated biodegradable multi-functional biocomposite films possess various imperative properties, making them ideal for utilization as packaging material.

Sujosh Nandi and Proshanta Guha, 2018, in their experimental study on Preparation and Properties of Cellulose Nanocrystal-Incorporated Natural Biopolymer, have reported that, the chemical pretreatment followed by mineral acid hydrolysis has been preferred over the years for the production of CNC. However, the process, particularly acid hydrolysis, is not suitable for commercial scale because of high processing cost, difficult-to-discard hazardous effluent and maintenance of reactors. Therefore, ionic liquids (IL) have recently been projected as a promising substitute of the strong mineral acid, while low-boiling point of ILs has

increased its acceptance among the researchers. Consequently, extensive research is required to choose a suitable IL and optimize the processing conditions. The incorporation of CNC into different biopolymers such as starch, chitosan, rubber, and protein are reported to enhance the mechanical and barrier properties. However, a contradictory review is reported for barrier properties, particularly for water vapor permeability. The probable reasons would be the similar chemical structure of natural biopolymer and CNC, and inappropriate dispersion of CNC throughout the casted films. The inappropriate dispersion occurs because of agglomeration of CNC into polymer matrix which may reduce the tortuosity. Therefore, the interaction between polymer matrix and filler becomes an important area needed to be studied well in future. Furthermore, an interaction of nano-biocomposite film with food, real-time quality changes of packed foods, and mathematical modelling on how CNC disperses into polymer matrix could be the areas needed to be explored in future to understand the effect of interaction among natural biopolymer and CNC.

Chen *et al*., 2016, have examined that, nanocellulose can be successfully isolated from a new, renewable, low-cost and abundant natural source, *Gelidium elegans* red algae biomass via alkalization, bleaching treatment and acid hydrolysis treatment. The physicochemical characterization results showed that the crystallinity index of isolated nanocellulose was 73 % with an average fibrils diameter of 21.8 ± 11.1 nm and average length of 547.3 ± 23.7 nm. Thus, the corresponding aspect ratio of isolated nanocellulose was about 25.TGA study showed that the neutralized nanocellulose products exhibited in better thermal stability than the untreated and other chemically-treated fibers. Due to its high crystallinity and better thermal stability, the isolated Gelidium cellulose nanoparticle is a new source and has great potential for various applications such as reinforcement agent in nanocomposites manufacturing and nano-fillers for polymer matrices.

In 2019, Huijing Chen *et al*., have extracted sulfated agar from *Gracilaria lemaneiformis* using hydrogen peroxide-assisted enzymatic method and analysed that to avoid the alkali residue pollution during the alkaline extraction process and to deal with the problem of difficult filtration during enzymatic extraction of sulfated agar, a “green” extraction called H2O2-assisted enzymatic method was developed. Agar was successfully extracted from *G. lemaneiformis*, and the sulfate content of EHA was higher than that of AA. Moreover, the filtration efficiency and dehydration rate of EHA improved due to the decreased viscosity of extracts, covering the shortcomings of the enzymatic extraction process. Compared with AA, EA and EHA exhibited higher extraction yield and lower melting and dissolution temperatures.

Furthermore, from the result of ESI-TOF-MS analysis, the agar with high sulfate content can be prepared by H2O2-assisted enzymatic method. The results obtained from this study could provide useful information for the development of sulfated polysaccharides in food, pharmaceutical, cosmetic, and biotechnological application.

Naturally activated edible films with antioxidant properties prepared from red seaweed *Porphyra columbina* biopolymers was developed by Raúl E. Cian *et al*., 2013, and have observed that, PF enriched in phycobiliproteins, is discarded during phycocolloids extraction. However, the excellent antioxidant properties of this fraction made it a potential byproduct. Moreover, PcF, mainly composed by phycocolloids, extracted from red seaweed without an ulterior purification steps gives a low cost potential source of hydrocolloids for producing an edible film. In this sense, it was possible to prepare edible films naturally activated with antioxidant properties from mixtures of both fractions. In particular, PcF films had excellent mechanical properties and PF films showed important antioxidant capacity. Films prepared by mixing different proportions of PF:PcF fractions showed intermediate properties which correlated with its formulation. Taking into account that the antioxidant activity of phycobiliproteins and phenolic compounds was preserved during film forming processing (drying step), these natural polymeric matrices may be evaluated for protecting other bioactive compounds added to any specific purpose.

According to the study conducted by Nan Wu *et al*., 2013, on Production and Rheological Studies of Microalgal Extracellular Biopolymer from Lactose Using the Green Alga *Neochloris oleoabundans*, it was analysed that, *Neochloris oleoabundans* was shown to be able to produce large quantities (up to 5 g/L) of high viscosity polymers with a weight-average molecular weight of 505 kDa from lactose under mixotrophic cultivation conditions. Aqueous solution of the polymers showed typical rheological behaviours of pseudoplastic fluids. The viscosity of the solutions was shown to be shear rate sensitive and temperature dependant. Adding 0.3–0.6 M NaCl into the aqueous solutions led to significant increase of viscosity. These properties suggest that the polymer could be utilized in a variety of fields including food, cosmetic, and oil industries.

# MATERIALS AND METHODS

## Study Site

Kerala has a coastline of about 580 km, which is extended in 9 districts of the state from Poovar, Thiruvananthapuram district in south to Thalapady, Kasaragod district in north. The coast of Kerala supports a large number of marine flora and fauna, owing to its variety of habitats such as beaches, back waters, estuaries, cliffs, lagoons, mangroves and coral reefs. Thus it forms an integral part of the marine biodiversity of India. *Gracilaria corticata,* the sample for our study was collected from coast of Thikkodi beach in Kozhikode district. The beach has latitude of 11° 28' 20.8" N and a longitude of 75° 37' 04.5" E. In this area rocks of different kinds and granite stones are found in the intertidal and subtidal region with luxuriant growth of various green, brown and red algae of few seaweed vegetation is found on them.

## Collection of Sample, Preservation and Processing

The seaweed sample was collected randomly from the intertidal regions, during the low tides by hand picking and use of knife. The collected sample was washed thoroughly in seawater and stored in polythene bags along with seawater for delaying degradation during the transport from the collection site to the laboratory. The sample was identified and confirmed as *Gracilaria corticata* by reference of literature (Srinivasan, 1973; Desikachary *et al*., 1998; Jha *et al.,* 2009) (Fig. 1). In the laboratory, the sample was washed under running water, all the surface debris as well as other smaller organisms was removed. For preparing the dried samples, the specimen was kept under shade for 6-7 days and finally dried in hot air oven. It was then powdered and used for further processes.

## Extraction of polysaccharides from *Gracilaria corticata*

100 g of the milled *G. corticata* was treated with 1L isopropyl alcohol (80% w/v) under constant mechanical stirring overnight at room temperature to remove pigments, lipids, some phenols, and low molecular weight compounds. To separate the sediment from the solvent (isopropyl alcohol), a refrigerated centrifuge with the controlled temperature at 10°C; 8,000 rpm for 10 minutes was applied, and then the supernatant was discarded.

The residual was rewashed with isopropyl alcohol (80% w/v), rinsed with acetone, and centrifuged at 10,000 rpm (10°C) for 10 minutes again, and dried at room temperature in a fume hood overnight. To extract the polysaccharides, 20 g of de-pigmented powder was added to 500 mL distilled water and the extraction was carried out at 65 °C with a stirrer for 2 hours. The supernatant was collected after centrifugation at 10°C and 10,000 rpm for 10 minutes, and the extraction was conducted twice. The supernatant was concentrated using the rotary evaporator under reduced pressure at 60°C. The concentrated extract was frozen at −20°C prior to lyophilisation to obtain the dried form of polysaccharides (Fig. 2).

**Characterization of the extracted polysaccharide**

**Determination of Physical properties**

**Solubility**

The solubility of the isolated polysaccharides was tested according to the method of Wheet (2011). About 1 mg of pure compound was added into 5 small test tubes. Different solvents such as DMSO, water and ethanol added separately. The solubility of the compound in each of these solvents was observed and recorded.

**Fourier Transform Infra-Red spectrophotometry (FT-IR)**

Fourier-transform infrared (FT-IR) spectra were recorded using a Thermoscientific Nicolet iS50 FT-IR spectrometer, catalogue number 912A0760. It has got a spectral range of 15 to 27,000 cm-1 and has an automated beam splitter exchanger (ABX) which can easily detect varies bond formation.

**X-ray diffraction (XRD) analysis**

XRD was performed with an Aeris Benchtop X-ray Diffractometer Malvern PANalytical to investigate the phase and crystallinity of the extracted polysaccharide powder. The XRD patterns were recorded in the region of 2θ from 0° -80° (Liang and Wang, 2017). The crystallinity of the molecules was calculated using the following equation.

$$\text{Crystallinity percentage} = \frac{Total\ area\ of\ crystalline\ peak}{Total\ area\ of\ all\ peaks} \times 100$$

### *In vitro* antioxidant activity

To different volume of extract, 0.5 ml of 1 mM ethanolic solution of DPPH was added and made up to 2.0 ml using ethanol. The mixture was allowed to react at room temperature for 30 minutes. Ethanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer. The assay was calculated as:

Radical scavenging activity = $\frac{Control-Test}{Control} \times 100$

## Nanosilica synthesis from Rice husk

Nanosilica is synthesized by sol-gel method from rice husk. The chemical reaction for the precipitation of silica involved:

$$SiO2 + 2NaOH \rightarrow Na2SiO3 + H2O$$

$$Na2SiO3 + 2HCl \rightarrow SiO2 + 2NaCl + H2O$$

**Washing and drying**: Rice husk was washed thoroughly with water to remove the soluble particles, dust, and other contaminants. It was then dried in an air oven at about 110°C for 24 hours.

**Thermal treatment:** The washed rice husk was weighed and subjected to heat treatment to obtain the ash. Sample was burned inside a programmable furnace at 700°C for 6 hours.

**Acid treatment:** Acid washing step was done to remove the small quantities of minerals prior to silica extraction from rice husk ash (RHA). Ten grams of RHA sample were dispersed in 60 ml of I N HCl for 5 minutes. It was then washed thoroughly.

**Silica extraction**: A sample of 2.5 g RHA was stirred in a 250 ml of 0.5N sodium hydroxide solution. The solution was heated in a covered beaker by stirring constantly, allowed to stand at room temperature and then filtered.

**Nanosilica preparation**: HCl was added until neutralized. The precipitate silica was washed repeatedly with warm, deionized water and then centrifuged at 5000 rpm for 10minutes. It was repeated for 3 times to obtain a neutral pH. The product was dried at 110°C for 24 hours in the

oven and crystallization in a programmable furnace at 450°C for1 hour. The obtained silica was crushed and preserved until further experiments (Figure 3).

**Characterization of Silica nanoparticles**

**X-ray diffraction (XRD) analysis**

XRD was performed with an Aeris Benchtop X-ray Diffractometer Malvern PANalytical to investigate the phase and crystallinity of the Nano-silica particles, of which the XRD patterns were recorded in the region of 2θ from 0° -80° (Liang and Wang, 2017). The crystallinity of the molecules were calculated using the following equation

$$\text{Crystallinity percentage} = \frac{Total\ area\ of\ crystalline\ peaks}{Total\ area\ of\ all\ peaks} \times 100$$

**Preparation of Biodegradable bioplastic for food packaging**

The bioplastic preparation was carried out by trail and error method by fixing the concentration of extracted polysaccharide powder at 1.5% (w/v) and varying the concentration of silica nanoparticles and glycerol from 1% to 7% (w/w of Polysaccharide powder) and 5% to 15% (w/w of Polysaccharide powder) respectively. Potential antioxidant agent, Ascorbic acid, was added to incorporate antioxidant property to the bioplastic at a concentration of 50% of w/w of silica nanoparticles. The three components in appropriate concentrations along with the essential oil were mixed and stirred for 30 minutes at 90°C. Sufficient amount of the above mixture was poured in a clean oiled petri dish and kept in hot air oven at 60°C to 80°C for 3 hours for setting. The film was removed after proper drying and evaluated for various properties (Figure 4)

**Characterization of developed bioplastic film**

**Solubility ratio:**

The water solubility (WS) ratio was determined as per basic standard method reported earlier (Wang & Rhim, 2015; Arham *et al.*, 2016; Sanyang *et al.*, 2016). The sample was oven dried at 220 ºF for 24 hours and weighed (W1) properly. The dried pieces were then immersed in centrifuge tubes containing 30 ml distilled water and kept in water bath at 25ºC with slow

shaking for overnight. The solutions were filtered and the remnants on filter paper were dried at 220 °F for two hours and reweighed (W2). The undissolved dry matter was calculated by using the formula given below.

Solubility (%) = $[(w1 - w2) \div w1] \times 100$

**Rate of biodegradability**

The developed bioplastic film pre-weighed (B1) and buried for a week in pots containing conditioned garden soil. The final weight (B2) of the film was recorded and the difference in the weight of the films was calculated by using the following equation (Hii *et al.,* 2016).

Biodegradability (%) = $[(B1 - B2) \div B1] \times 100$

***In vitro* antioxidant activity**

To different volume of extract 0.5 ml of 1 mM ethanolic solution of DPPH was added and made up to 2ml using ethanol. The mixture was allowed to react at room temperature for 30 minutes. Ethanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer. The assay was calculated as:

Radical scavenging activity = $\frac{Control - Test}{Control} \times 100$

**(a)Fresh specimen**

**(c) A single filament**

**(b) Dry specimen**

**Figure 1:** ***Gracilaria corticata***

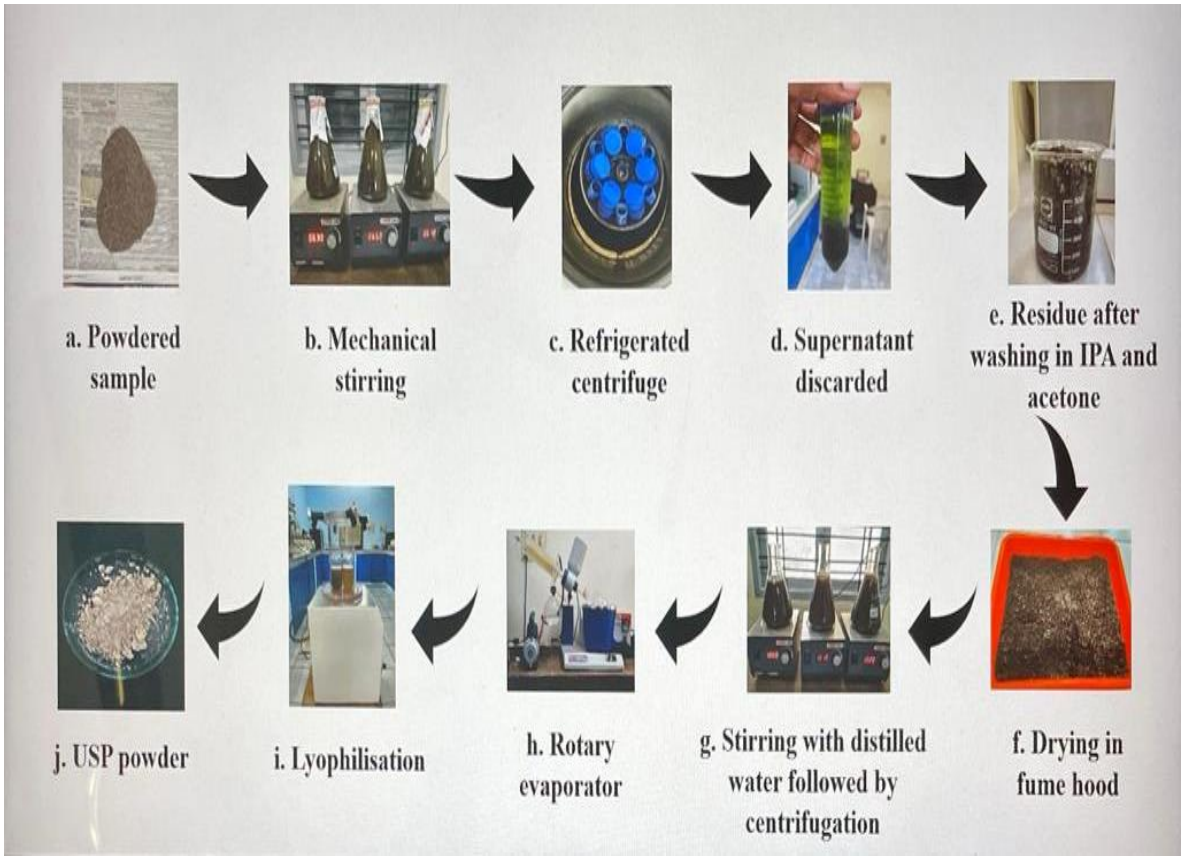



**Figure 2: Extraction of Polysaccharide**

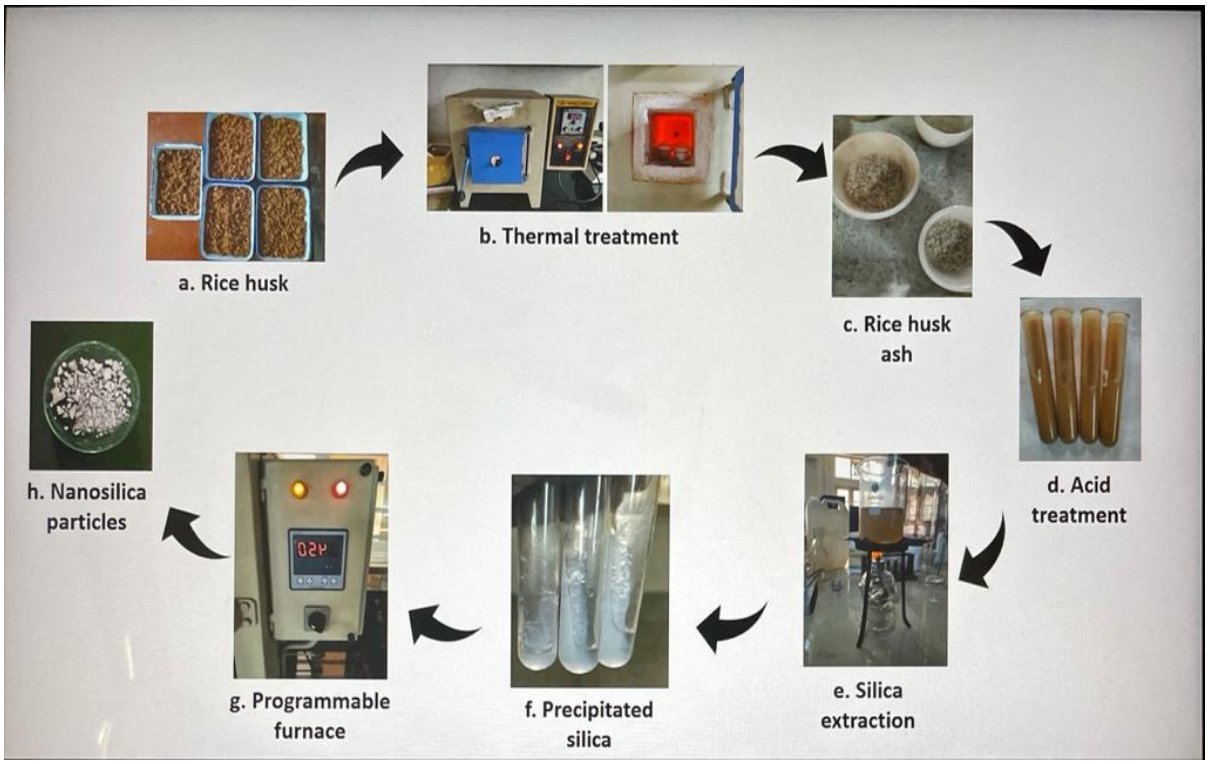



**Figure 3: Extraction of Nanosilica particles**

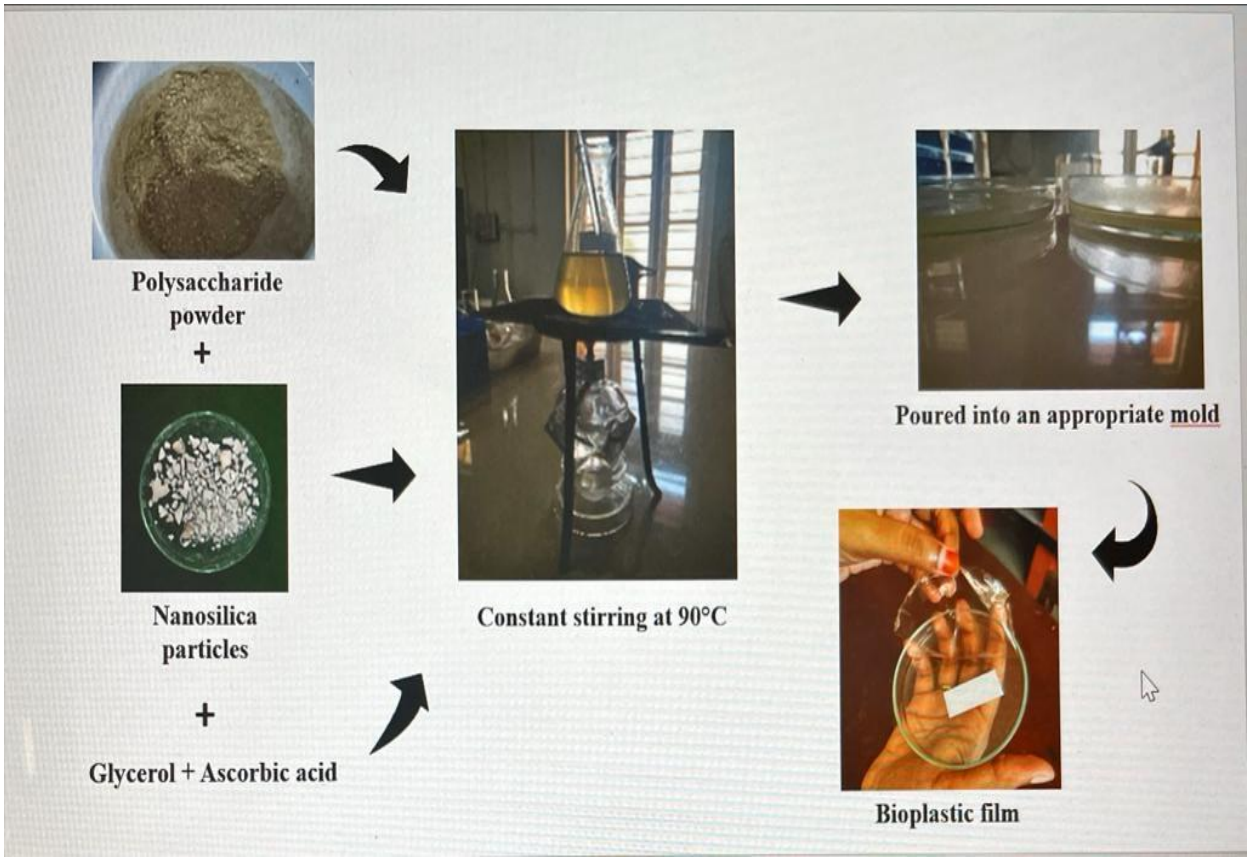



**Figure 4: Preparation of Bioplastic film**

# OBSERVATION AND RESULT

## COLLECTION OF FRESH SPECIMEN

Around 7Kg of Fresh algal specimen, *Gracilaria corticata*, was collected from Thikkodi coast of Kozhikode district

## DRYING AND PRESERVING

Algal specimen was washed thoroughly, shade dried for 7 days and was powdered to obtain 420g of powdered sample.

## EXTRACTION OF POLYSACCHARIDES FROM GRACILARIA CORTICATA

Polysaccharide was extracted from powdered sample of seaweed, *Gracilaria corticata* by cold extraction method.

The yield thus obtained was measured as, 19.99g of USP powder (freeze-dried powder) (as shown in Figure 10) of polysaccharide from 50g of the algal sample. Therefore, 39.98% of polysaccharide USP powder was obtained with respect to the algal sample taken.

## CHARACTERIZATION OF THE EXTRACTED POLYSACCHARIDE

### Determination of Physical properties

### Colour and solubility:

Polysaccharide powder extracted was observed to be ivory in colour. Solubility of the sample was tested in different solvents, and was found to be soluble in water, ethanol and DMSO (Dimethyl sulfoxide).

**Fourier transform infrared (FT-IR) spectroscopy**

FT-IR spectra of agar showed vibrational peaks at 3362.42 cm-1 which indicates the presence of O-H stretching and the corresponding broad peak is around 3400cm-1 due the hydroxyl groups of polysaccharides and hydrogen bonding. In 1000cm-1 there is CH bending (Figure 5). It is also observed that the presence of band at 989.94 cm-1 1040.27 cm-1 and 1094.56 cm-1 indicates the presence of anhydrous groups.

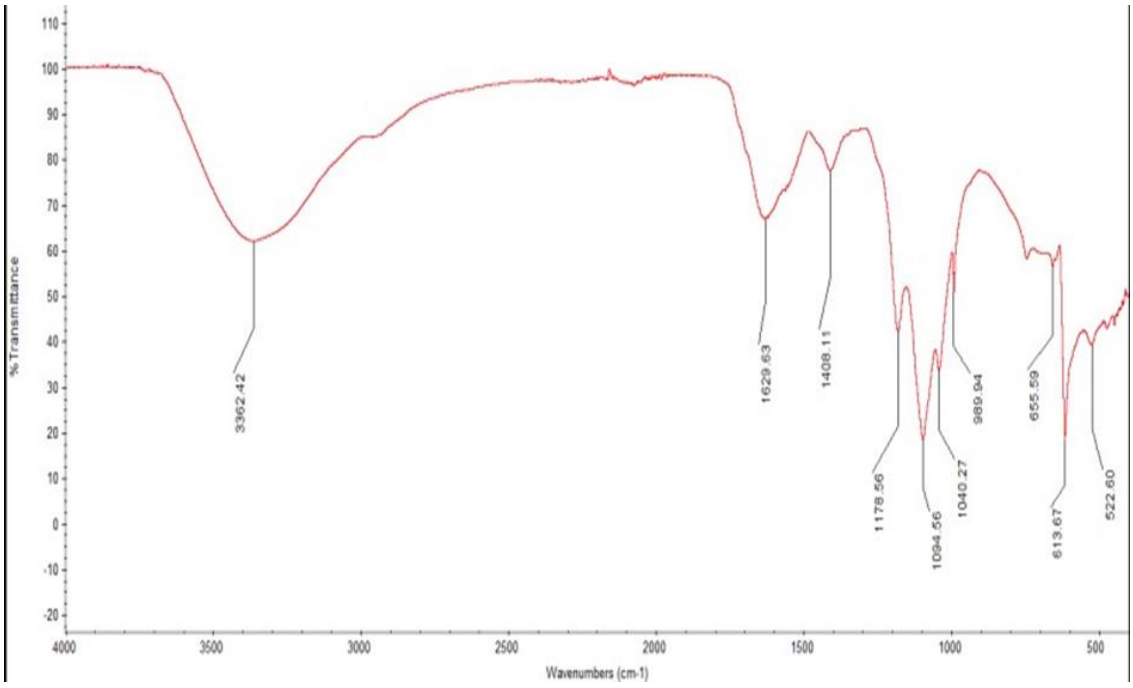



**Figure 5**

**X-ray diffraction (XRD) analysis:**

The polysaccharide powder was found to be semi crystalline in nature with a 55.38% crystallinity and four major peaks were identified using the software OriginLab (Figure 7)

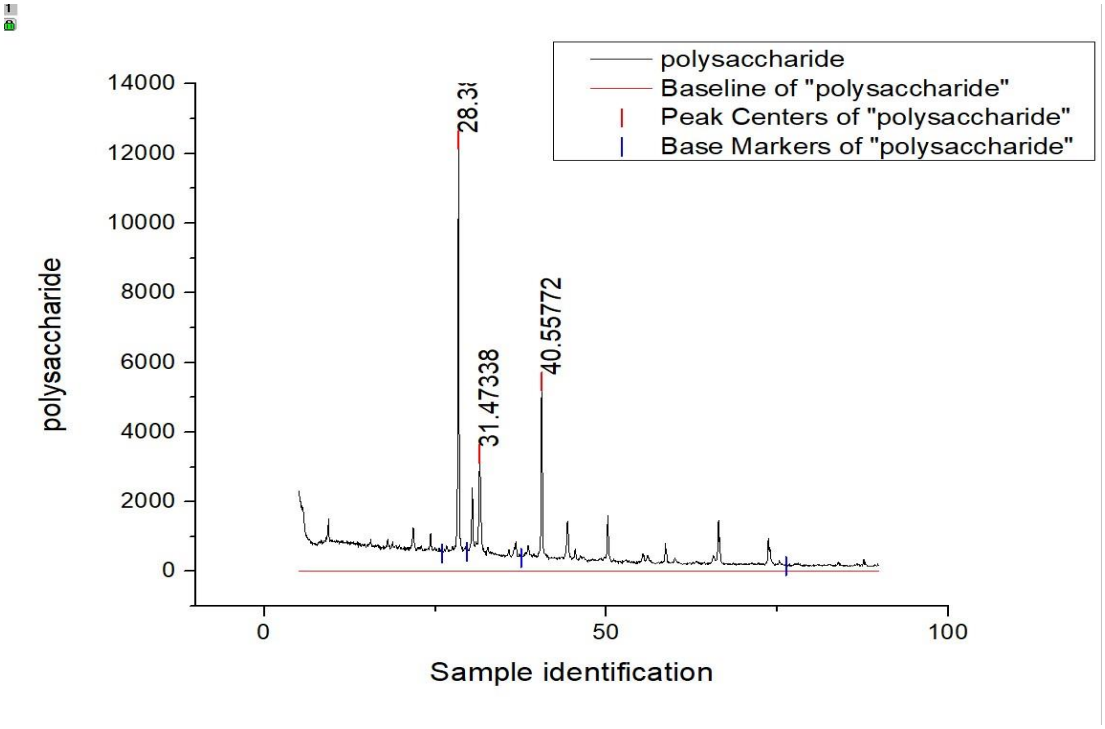



**Figure 6**

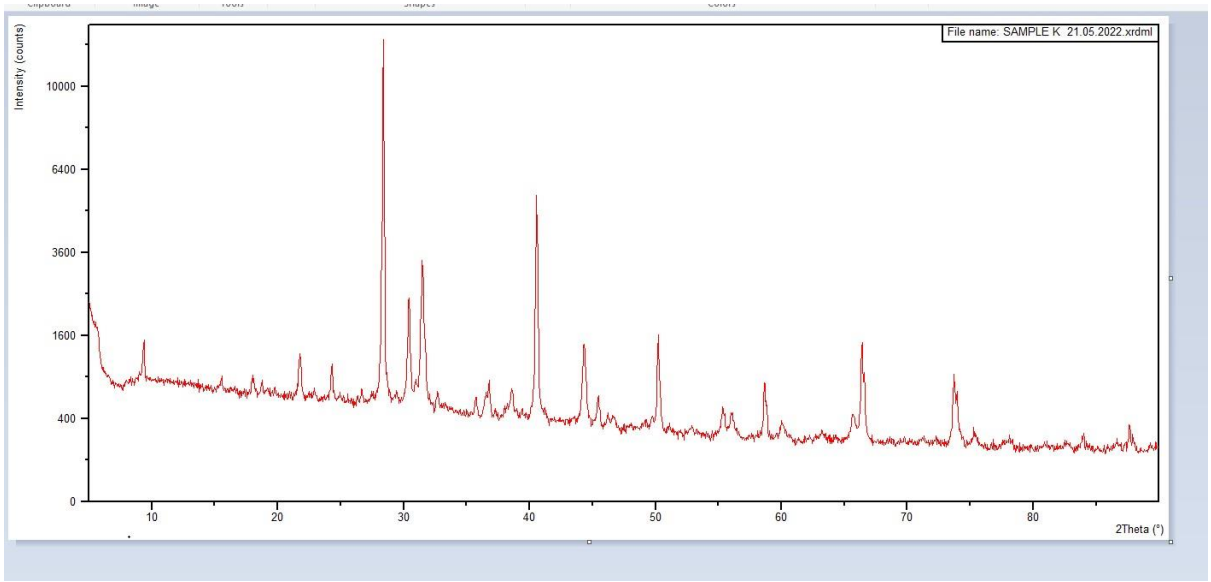



**Figure 7**

***In vitro* antioxidant activity:**

By using DPPH assay (2,2-diphenyl-1-picryl-hydrazyl-hydrate radical scavenging assay), the scavenging activity (% of inhibition) of the polysaccharide at different volumes on the odd electron (free radical) of nitrogen atom of DPPH reagent, was found to be very less as per the data in table 1 obtained by applying the following equation;

$$\text{Radical scavenging activity (\% of Inhibition)} = \frac{\text{OD value of control} - \text{OD value of test solution}}{\text{OD value of control}} \times 100$$

Where, OD value of control was obtained as, 0.436 at 518nm

Concentration of the sample of polysaccharide= 100 mg/ml

| **Volume of sample (µl)** | **OD value at 518 nm** | **% of Inhibition** |
|---|---|---|
| 100 | 0.418 | 4.12 |
| 200 | 0.402 | 7.79 |
| 300 | 0.389 | 10.7 |

**Table1**

Although, % of inhibition increases as volume of the sample increase, only a poor scavenging activity of 10.7% was observed, even for the highest volume of sample taken for the assay. Low scavenging property of the polysaccharide could be due to, variations in various factors affecting antioxidants, like polysaccharide conjugates, polysaccharide mixture in crude polysaccharide extracts, polysaccharide chelating ions, metal ion-enriched polysaccharides, chemical modifications of polysaccharides and structural features of polysaccharides, etc. as reported by Mohanta *et al*., 2022.

**NANOSILICA SYNTHESIS FROM RICE HUSK**

Crystalline form of Nanosilica particles were extracted from Rice husk by using Sol-gel method of extraction. Colour of the powder was observed to be pure white. A yield of 16.88g of nanosilica particles (as shown in Figure 11) were obtained from 120g of rice husk, which was determined as, 14.06% of the rice husk.

## CHARACTERISATION OF NANOSILICA PARTICLES

### X-ray diffraction (XRD) analysis

The Nanosilica particles extracted was found to be highly crystalline in nature. Four major peaks were identified with the help of a software called OriginLab (Figure 9), in the XRD graph (Figure 8), obtained by performing the analytical test. From the graph, it was discovered that the nanosilica particles extracted were 98.43% crystalline.

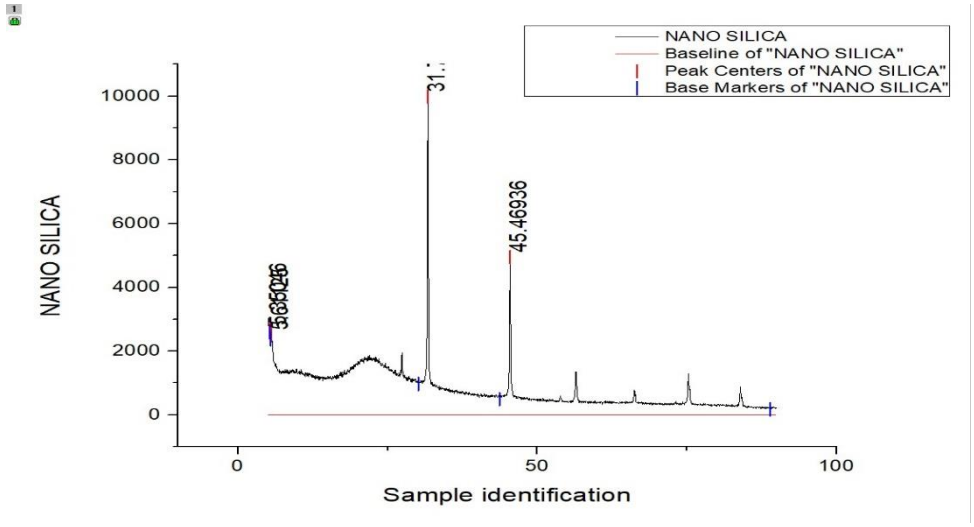



**Figure 8**

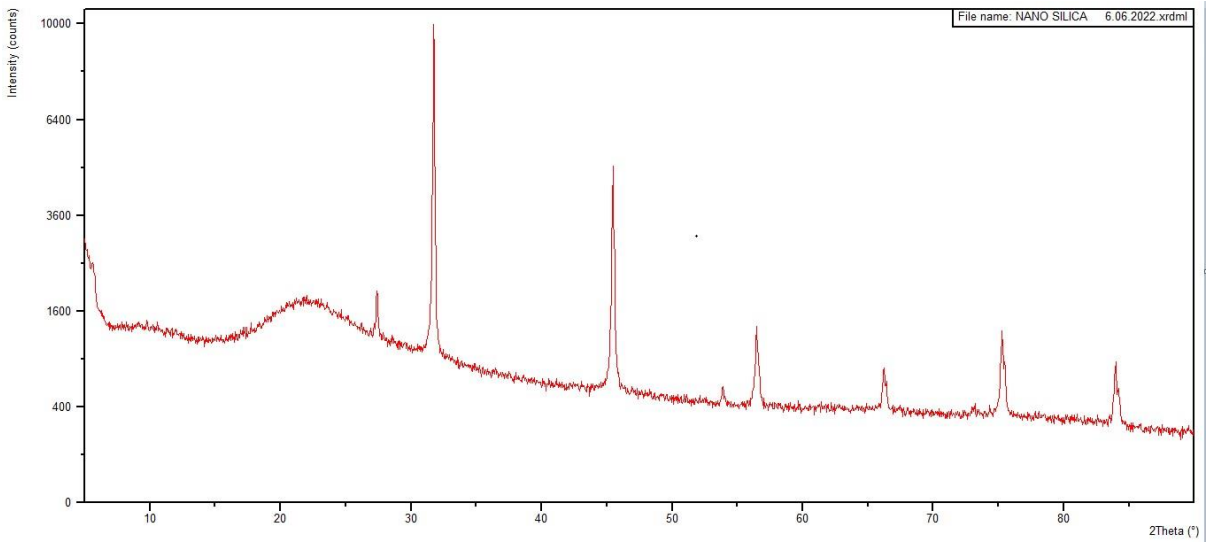



**Figure 9**

## PREPARATION OF BIODEGRADABLE BIOPLASTIC FOR FOOD PACKAGING

The bioplastic preparation was carried out by trial and error method by fixing the concentration of extracted polysaccharide powder at 1.5% (w/v). A concentration of 5% (w/w of polysaccharide) of extracted Nanosilica particles and 11% (w/w of polysaccharide) of Glycerol were selected as the ideal concentrations for the preparation of mechanically stable, non-brittle, biodegradable bioplastic film with uniform thickness (as shown in Figure 11)

Antioxidant property was incorporated to the biofilm by the addition of 50% (w/w nanosilica particles) of a potential antioxidant agent, Ascorbic acid into the film formation mixture.

## CHARACTERIZATION OF DEVELOPED BIOPLASTIC FILM

### Solubility ratio:

Solubility of the prepared bioplastic film in distilled water, by initial stirring and keeping for overnight, was calculated by the formula;

Solubility (%) = $[(w1 - w2) \div w1] \times 100$

Where, w1 (Initial weight) = 0.0223g

w2 (Final weight) = 0.0190g

Therefore, Solubility % = $\frac{0.0223 - 0.0190}{0.0223} \times 100$

Solubility % = 14.79%

The solubility % obtained for the biofilm as 14.79 %, can be considered as a supportive element for the biological nature of the biofilm. At the same time, bioplastics having low grade solubility are considered to be the best, as they resist moisture for a longer period of time and helps to increase the shelf life of the product (refer Figure 13).

### Rate of biodegradability:

Biodegradability rate of the prepared bioplastic film was determined by burying the film in garden soil for 1 week, and the numerical value was measured by using the formula;

Biodegradability (%) = [(B1 – B2) ÷ B1] × 100

Where, B1 (Initial weight) = 0.0389g and B2 (Final weight) = 0.0348g

Therefore, Biodegradability % = $\frac{0.0389 - 0.0348}{0.0389} \times 100$

Biodegradability % = 10.54%

The rate of biodegradability obtained for the synthesised bioplastic film as 10.54% in one week, is examined as a shelf life quality enhancing property of the film, due to the incorporation of antimicrobial and antioxidant potential agent, Ascorbic acid into it. Hence, rapid and early contamination of the biofilm by invading microbes can be controlled, and shelf life for few days, can be achieved for food packaging (refer Figure 14)

***In vitro* antioxidant activity:**

By using DPPH assay (2,2-diphenyl-1-picryl-hydrazyl-hydrate radical scavenging assay), the scavenging activity (% of inhibition) of the synthesised bioplastic film, at different volumes on the odd electron (free radical) of nitrogen atom of DPPH reagent, was found to be great, as per the data in table 2 obtained by applying the following equation;

Radical scavenging activity (% of Inhibition) = $\frac{\text{OD value of control} - \text{OD value of test solution}}{\text{OD value of control}} \times 100$

Where, OD value of control was obtained as, 0.685 at 518nm

Concentration of the sample of biofilm = 100 mg/ml

| **Volume of sample (µl)** | **OD value at 518 nm** | **% of Inhibition** |
|---|---|---|
| 100 | 0.356 | 48 |
| 200 | 0.295 | 56.9 |
| 300 | 0.234 | 65.8 |

**Table 2**

An increase in % inhibition, as the volume of the sample increases, denotes a good rate of antioxidant property of the biofilm, eventhough a minimal concentration (50% w/w of nanosilica particles = 0.0375g) of a potential antioxidant agent, Ascorbic acid, was incorporated into the biofilm.

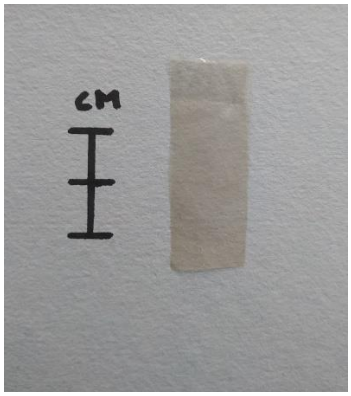
**Figure 10: Polysaccharide**

**Figure 11: Nanosilica**

**(a)**

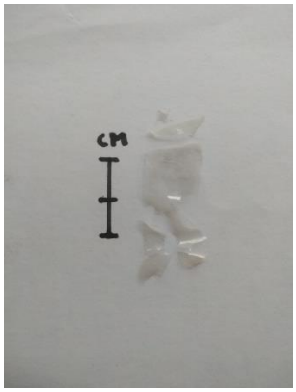
**(b)**

**Figure 12: Bioplastic film**



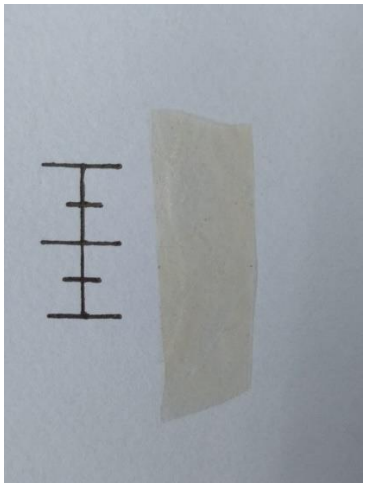

(a) Initial state of biofilm

(b) Biofilm in distilled water



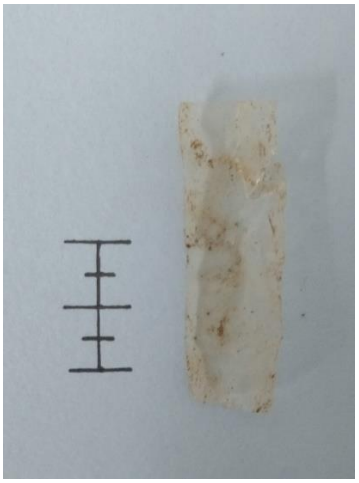

(c) Final state of

**Figure 13: Solubility of biofilm**



(a) Initial state of biofilm

(b) In soil Biodegradability



(c) Final state of biofilm

**Figure 14: Biodegradability of biofilm**

# DISCUSSION

The principal roles of food packaging are to protect food products from physical, chemical, and biological influences by delaying food deterioration, retaining and prolonging the beneficial effects of processing, and maintaining the quality and safety of the foods with extending shelf life (Marsh and Bugusu, 2007). Non-renewable, non-biodegradable packaging materials have serious environmental drawbacks. They have been considered as a major source to the solid waste and environmental pollution by consumers and environment activists (Risch, 2000; Ramos *et al*., 2013). In order to solve this problem, companies and researchers have been working on ways to develop new packaging strategies with environmentally friendly, abundant biodegradable packaging materials made from renewable natural polymers (Risch, 2000; Gontard and Guilbert, 1994).

The present study, has a positive impact on food packaging system, in which, a biodegradable edible film was synthesised as an alternative to synthetic polymer containing packaging materials. Biofilms, wrapping food products, as the primary barrier against physical impacts, prevent contamination, increase shelf life and contain important information about packaged food. In addition, biofilms can be used to improve food quality, as they can carry functional ingredients such as fatty acids, antioxidants, antimicrobials, nutrients, and flavors to further enhance food quality, stability, functionality, and safety (Lin and Zhao, 2007).

*Gracilaria corticata* was selected as the sample for the study. It was collected from coast of Thikkodi beach in Kozhikode district. The seaweed sample was collected randomly from the intertidal regions, during the low tides by hand picking and use of knife. A very rich collection of the algal sample was obtained during the collection process, which was done in the month of January. Therefore, this season can be considered as a favourable time for the collection of this species of *Gracilaria,* which is in accordance with the finding of Rosemary *et al*., 2019, that has reported, Gracilaria edulis and G. corticata is abundantly available in almost all seasons in Palk Bay, on the southeast coast of India, rather than other Gracilaria sp. Both *G. edulis* and *G. corticata* are commercially important and commonly edible seaweeds in India.

Biodegradable edible film was prepared by taking polysaccharide extracted from *Gracilaria corticata*, as the biopolymeric backbone. Extracted polysaccharide was obtained as a USP powder, of ivory colour. Cold extraction method was adopted which gave a better yield of polysaccharide (39.98% of algal sample) as compared to less yield (27.2% of *Gracilaria*

*birdiae*) obtained by Rosemary *et al*., 2019, when the extraction process followed was hot extraction type. However, in polysaccharide extraction conducted by Syad *et al*., 2013, lower carbohydrate content in Gracilaria species, such as *G. acerosa*, was reported (1.05 g/100 g). Armisen in 1995, reported that the polysaccharide yield from Gracilaria species varies due to seasonal variations, physiochemical factors, environmental conditions and extraction methods. Additionally, as per Melo *et al*., 2002, report, the variations in the polysaccharide content of Gracilaria can vary depending on atmospheric temperature at the time of extraction.

Solubility of the polysaccharide sample was tested in different solvents, and was found to be soluble in water, ethanol and DMSO (Dimethyl sulfoxide), whereas, the polysaccharide extracted from Madagascan *Gracilaria corticata*, by Andriamanantoanina *et al*., 2007, was dissolved in aqueous solution of 0.1 M KCl during their study. Fourier-transform infrared (FT-IR) spectra of obtained polysaccharide were recorded, using a Thermoscientific Nicolet iS50 FT-IR spectrometer and the composition of the polymer was determined. In X-ray diffraction (XRD) analysis, the polysaccharide powder was found to be semi crystalline in nature with a 55.38% crystallinity. But in general, Polysaccharides are amorphous and tasteless carbohydrates that are insoluble in water. The biodegradability behaviour and the mechanical properties of polysaccharides are significantly controlled by their crystalline content and the crystallite size of crystals. Semi-crystalline polysaccharides have been utilized to reinforce the polymer composites for various applications ranging from packaging uses to biomedical applications as per the report given by Yazdi *et al*., 2021.

A poor antioxidant property was observed in the polysaccharide extracted from *Gracilaria corticata*, which could be due to, variations in various factors, like polysaccharide conjugates, polysaccharide mixture in crude polysaccharide extracts, polysaccharide chelating ions, metal ion-enriched polysaccharides, chemical modifications of polysaccharides and structural features of polysaccharides, etc. as reported by Mohanta *et al*., in 2022. Recently, seaweed polysaccharides have been given large attention by the scientific community due to their outstanding bioactivities and correspondingly low toxicity (Ju *et al*., 2019). They have been shown to have other beneficial health effects, including their prebiotic effect and antioxidant or anti-inflammatory activity (Francavilla *et al*., 2013). In a study conducted by Souza *et al*., 2012, the antioxidant properties of *Gracilaria birdiae*, sulfated polysaccharide were evaluated by measuring DPPH free-radical scavenging effect, showing that this polysaccharide has a moderate effect in inhibiting the formation of free radicals.

Crystalline form of Nanosilica particles were extracted from Rice husk by using Sol-gel method of extraction. Colour of the powder was observed to be pure white. Nanosilica particles yield was obtained as, 14.06% of the rice husk. A good yield of nanosilica particles, of white colour, were obtained by Amutha *et al*., 2010, when silica extraction method was applied on rice husk. X-ray diffraction (XRD) analysis of these particles, determined the morphological nature of the powder as amorphous in contrast to the highly crystalline nature of the nanosilica particles extracted using sol-gel method with 98.43% crystallinity. These extracted nanosilica particles were incorporated into biodegradable bioplastic film to improve their mechanical properties and provide strength to the film. Nanosilica reinforced bioplastic creates a bond between them, which is found to have increased thermal stability than the starch glycerol bond in raw bioplastic film. Nano reinforced bioplastic showed lower weight loss and higher heat capacity than raw bioplastic samples.

The bioplastic preparation was carried out by trial and error method by fixing the concentration of extracted polysaccharide powder at 1.5% (w/v). An ideal concentration of nanosilica particles, which improve mechanical properties and a plasticizing agent, Glycerol, to promote the plasticity and flexibility and to reduce the brittleness were added to prepare a mixture for the production of mechanically stable, non-brittle, biodegradable bioplastic film with uniform thickness. The film thus obtained was colourless, transparent and had a rough texture due to the presence of incorporated nanosilica particles. A solubility % of 14.79 % was obtained for the biofilm, which can be considered as a supportive element for the biological nature of the biofilm. At the same time, bioplastics having low grade solubility are considered to be the best, as they resist moisture for a longer period of time and helps to increase the shelf life of the product. Soil burial test was performed to analyze the level of deterioration caused to the bioplastics via microbial growth when discharged into the soil (Hii *et al*., 2016; Wahyuningtiyas & Suryanto, 2017). Then the rate of biodegradability was obtained as 10.54% in one week, which is same as in the percentage biodegradability of biofilm prepared by Marium Asif *et al.,* 2021, due to the addition of glycerol as the plasticizing agent, having hydrophilic properties (Vieira et al., 2011; Hii et al., 2016) and maximum moisture retaining properties which enable the microbial growth. It was then inferred as a shelf life quality enhancing property of the film, due to the incorporation of antimicrobial and antioxidant potential agent, Ascorbic acid into it. Hence, rapid and early contamination of the biofilm by invading microbes can be controlled, and shelf life for few days, can be achieved for food packaging.

Packaging plays a very important role in food preservation and in health-enhancing foods (Cerqueira *et al*., 2010). Food packages usually act as inert barriers for product protection with no interaction with food. But nowadays, most packaging materials are derived from nonrenewable sources. These synthetic polymers have been used as packaging materials for many years due to their economic and technological advantages, such as high availability, low cost, and favorable functional properties. However, such polymers present hydrophobic nature, which limits the action of microorganisms, and therefore takes many years to decompose. This results in the production of large volumes of solid wastes, which leads to serious environmental problems (Tharanathan, 2003). A total of 1.3 billion tonnes of municipal solid waste per year was generated in 2012, but it is expected to increase to 2.2 billion tonnes per year by 2025. Non-renewable, non-biodegradable packaging materials have serious environmental drawbacks. They have been considered as a major source to the solid waste and environmental pollution by consumers and environment activists (Risch, 2000; Ramos *et al*., 2013). These issues have been greatly aggravated due to the increase in population and economic growth from developed and developing countries. Hence, the need to reduce the amount of discarded plastics is being recognized globally, aiming to replace them with packaging films based on biodegradable materials, which are recognized as environmentally friendly materials (Tharanathan, 2003). However, edible films can provide additional protection for food, while being a fully biodegradable and environmentally friendly packaging system (Morales-Jiménez *et al*., 2020). Therefore, in this present work, a biodegradable bioplastic film was developed by using polysaccharide extracted from Gracilaria corticata as the biopolymeric backbone. Nanosilica particles extracted from rice husk were incorporated to the film to improve its mechanical properties. Also, glycerol was used as a plasticizing agent to promote plasticity and flexibility and to reduce the brittleness of the film.

Advances in food packaging researches led to the development of active packaging, and intelligent packaging. Active packaging is a novel method used to prolong the shelf-life of perishable foods, maintain or improve the quality and safety of prepared foods due to its interaction with the product. Besides, active packaging has potential to replace the addition of active compounds into foods, reduce the movement of particles from packaging materials to food, and get rid of industrial processes that can cause the introduction of pathogenic microorganism into the product (Schaefer and Cheung, 2018). In addition, bioactive packaging contains antimicrobial agents that interact with biological molecules and may inhibit the growth of various microorganisms (Brockgreitens and Abbas, 2016)

Using oxygen scavenging or absorbing agents like Ascorbic acid in edible biofilms, offers several benefits, such as inhibiting the formation of microbial growth, maintaining the quality of lipid-containing foods (preventing rancidity), avoiding discoloration, and avoiding oxidation. One of the most important characteristics of the ascorbic acid is its reducing ability. In the presence of oxygen, ascorbic acid tends to oxidise with a strong result, especially in relation to catalyst metals, removing the environmental resources of oxygen. Furthermore, the ascorbic acid can react with free radicals, arresting the chain reactions that may provoke dangerous effects on microorganisms (Cerutti, 2006). It allows maintaining stable other important elements, such as vitamin A, E, folic acid and thiamine in organisms and foods (Mora – Gutierrez and Gurin, 2006).

In the present study a potential antioxidant agent, Ascorbic acid, was incorporated into the bioplastic film, to integrate antioxidant and antimicrobial properties to the film. Antioxidant study of the film was conducted by DPPH assay, and an increase in % inhibition was observed as the volume of the sample increases. This denotes a good rate of antioxidant property of the biofilm, eventhough a minimal concentration of Ascorbic acid was incorporated into the biofilm. Thus, antioxidant property integrated biodegradable bioplastic, with improved mechanical properties can be used as a promising alternative way for safe packaging of food.

# SUMMARY AND CONCLUSION

Food contact materials (FCMs) are materials that come in contact with food products such as food packaging which play a significant role in the food quality and safety. Plastic, which is a major food packaging material, harms the eco-system, wildlife, and the environment. As a result, numerous researches have been in progress on alternative polymers, which has similar properties as plastic but is also environmentally friendly (biodegradable). In recent years, the utilization of seaweed polysaccharides has piqued interest due to its biodegradability, non-toxicity, antioxidant capabilities, and excellent film formation ability (Perera *et al*., 2021).

In the present work on, preparation of bioplastic from *Gracilaria corticata* (J. Agardh) J. Agardh polysaccharides with nanosilica reinforcement, a biodegradable bioplastic edible film was prepared by using polysaccharide extracted from a red algae, *Gracilaria corticata*, as the biopolymer. Cold extraction process was carried out to obtain a good yield of USP powder of polysaccharide which was ivory in colour. Recently, seaweed polysaccharides have been given large attention by the scientific community due to their outstanding bioactivities and correspondingly low toxicity (Ju *et al*., 2019). They have been shown to have other beneficial health effects, including their prebiotic effect and antioxidant or anti-inflammatory activity (Francavilla *et al*., 2013). Characterisation of the extracted polysaccharide was done by analysing its solubility in different solvents, out of which, it was found to be soluble in water, ethanol and DMSO. Using Fourier-transform infrared (FT-IR) analysis, the composition of the polymer was discovered. Morphological nature of USP powder was determined as semicrystalline, by X-ray diffraction (XRD) analysis. A very poor antioxidant activity was shown by the polysaccharide in DPPH assay.

Nanosilica particles extracted from rice husk by sol-gel method was reinforced to the film mixture to improve its mechanical properties and provide strength to the film. Nanosilica reinforced bioplastic creates a bond between them, which is found to have increased thermal stability than the starch glycerol bond in raw bioplastic film. Nano reinforced bioplastic showed lower weight loss and higher heat capacity than raw bioplastic samples. A good yield of nanosilica particles, of white colour, were obtained. Highly crystalline nature of extracted silica was determined by X-ray diffraction (XRD) analysis.

An ideal concentration of glycerol was added to the film mixture as a plasticizing agent to promote plasticity and flexibility and to reduce the brittleness of the film. Advances in food

packaging researches led to the development of active packaging, and intelligent packaging. Active packaging is a novel method used to prolong the shelf-life of perishable foods, maintain or improve the quality and safety of prepared foods due to its interaction with the product (Schaefer and Cheung, 2018). Therefore, to integrate antioxidant property to the biofilm, Ascorbic acid, a potential antioxidant agent was added and its scavenging activity was measured using DPPH assay to obtain a high percentage of inhibition. After mixing up of all the required components in their ideal concentrations, a thin layer of the biofilm mixture was poured into an oiled petri dish for film formation. Solubility and biodegradability of the so formed biofilm was analysed. Less percentage of solubility and biodegradability of the film were considered to be the best, as they resist moisture for a longer period of time and helps to increase the shelf life of the product.

The utilization of seaweed in developing food packaging biofilms has sparked considerable scientific interest due to several beneficial characteristics of seaweeds such as biodegradability, non-toxicity, transparency, barrier properties, film-forming ability, and antioxidant /antimicrobial properties. The mechanical, chemical, physical, thermal, antioxidant, and antimicrobial properties of seaweed based biofilms have been substantially improved by using raw seaweed extract or its polysaccharide in combination with other biopolymers and additives such as nanoparticles and active agents. Active packaging system can be enhanced by nanosilica particles reinforcement, to improve its mechanical and thermal stability. Incorporation of a potential antioxidant agent provides protection against early degradation by extending shelf life. Therefore, this biodegradable bioplastic film synthesised, can be used as a potential and promising alternative way for safe and healthy food packaging system. Furthermore advanced properties, can also be added to the film easily, by incorporation of the property containing substance, to the film formation mixture, to enhance its quality at any time. These edible biofilm forming mixture, can also be applied as a thin outercovering / coating on fruits and vegetables to maintain their freshness, by providing protection against organisms causing early rotting and other damages. By promoting a prevalent use of biodegradable films, over synthetic polymer based packaging system, environmental pollution due to non-biodegradable waste disposal can be minimised.

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