Nanosilica reinforced bioplastic from *Gracilaria corticata* (J.Agardh) J.Agardh, an alternative to toxic counterparts

Dissertation submitted in partial fulfillment of the requirements
for the award of the degree of "**Master of Science**" in
BOTANY

By

C S KEERTHANA
Reg. No:AM20BOT007

DEPARTMENT OF BOTANY
ST. TERESA'S COLLEGE (AUTONOMOUS)
ERNAKULAM
2022

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CERTIFICATE

This is to certify that the dissertation entitled "**Nanosilica reinforced bioplastic from *Gracilaria corticata* (J.Agardh) J.Agardh, an alternative to toxic counterparts**" is an authentic record of work carried out by C S Keerthana 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.

Ms. Anu Joy
Supervising Teacher
Department of Botany
St. Teresa's College (Autonomous)
Ernakulam.

Dr. Liza Jacob
Head, Department of Botany
St. Teresa's College (Autonomous)
Ernakulam

Place: Ernakulam

Date:

External Examiners

1.

ACKNOWLEDGEMENT

It is my privilege to express my deep sense of gratitude to Ms. Anu Joy, Faculty, Department of Botany, St. Teresa's College (Autonomous), Ernakulam, for her valuable guidance, help and encouragement at every stage of this work.

I express my sincere thanks to Dr. Liza Jacob, Head of the Department of Botany, St. Teresa's College (Autonomous), Ernakulam for her advices and constant support extended throughout the work.

I am highly indebted to Dr. Pressy P Prakasia, Assistant Professsor, Department of Botany, All Saint's College, Trivandrum, for her vital guidance, which has promoted my efforts in all the stages of this project work.

I express my deep sense of gratitude to Dr. Jandas P J, Post doctoral fellow, Department of Applied Chemistry, Cochin University of Science and Technology, Ernakulam, Dr. E. A. Siril, Professor and Head of the Department of Botany, Kariavattom Campus, Trivandrum, and Vishnu B and Jayaraj, Research Scholar, Kariavattom Campus, Trivandrum, for their kind help and support in successful completion of my project.

I do thank all the teaching and non teaching staffs, Department of Botany for all the help rendered during the course of the study.

I thank my fellow project mates for stimulating discussions and also for all the fun we had together. I express my gratitude to my classmates who played an excellent role in showering friendly attitude, love and generous hospitality.

I express my heartfelt gratitude to my family members for supporting me directly or indirectly and giving me constant motivation in the completion of the project.

Finally, I thank the God almighty for guiding me the right path and bestowing me with great strength.

C S Keerthana

Introduction

Algae are ubiquitous; a multitude of species ranging from microscopic unicellular to gigantic kelps inhabit the world's oceans, freshwater bodies, soils, rocks, and trees, and are responsible for most of the global production of organic matter by photosynthesis (Hork *et al.,* 1995). Seaweed can be referred to as large marine algae that grow well in the shallow salty waters of the ocean and are visible when the sea tides are low. They play a fundamental role in the ecosystem, as they are the major producers of the biosphere. Algae are one of the oldest organisms present on earth, evolved millions of years ago. Hence they are used in many traditional home remedies, which have been handed down through generations. Often, these organisms are regarded as an under-utilized bioresource, although many species have been used as sources of food, industrial gums, and in therapeutic and botanical applications for centuries.

Marine macro algae or seaweeds have been used as food, especially in China and Japan, and crude drugs for treatment of many diseases such as iodine deficiency (Goitre, Basedow's disease, and hyperthyroidism). Some seaweeds have also been used as a source of additional vitamins and other nutrients, treatment of various intestinal disorders, as vermifuges, antiviral, antibacterial, antifungal, antitumoral, anti-inflammatory, anti-allergic, and antithrombotic, neuroprotective, hypocholesterolemic and hypoglycemic agents, and in thalassotherapy treatments. Seaweeds have been also employed as dressings, ointments, and in gynecology (Trease and Evans, 2009). It has been reported that seaweeds serve as an important source of bioactive natural substances (Smit, 2004). Many metabolites isolated from marine algae have been shown to possess bioactive effects (Faulkner, 2004). In fact, the discovery of metabolites with biological activities, from macroalgae, has increased significantly in the past three decades. On the other hand, seaweeds have recently received significant attention for their potential as natural antioxidants (Chandini *et al.,* 2008). Hence they are always been a valuable resource to man in the form of food as well as they provide raw materials for various industrial applications.

The major environmental threat that we face today is the rapid growth in the world population. Every year the population increases by 1.1 % which is around 81 billion people. The immediate effect of this condition is the aggregated need of space, food, water, energy, natural resources etc. To meet all the needs of this growing population, we are dependent solely on this environment. To make space for building shelter and land for cultivation, we clear out forest areas; to meet our energy requirements, we obtain various minerals like coal, lead, copper etc. from the deep underground ores through mining. These natural resources like the lush vegetation covers (the forest) or the mineral ores are formed as a result of millions of years biological and geological activities. Once they are destroyed or exhausted, building them back is not an easy task.

Apart from running out of resources, the other major crisis that we face is environmental pollution. Beginning from the 19^{th} century, more precisely when the world turned from an agricultural based economy to an industrial based economy, one of the major problems that evolved and still exist is pollution. The age of industrialization has slowly lead to the degradation of all aspects of the environment like air, water, soil etc. The various pollution causing agents are carbon monoxide, lead, nitrogen oxides, ground-level ozone, particle pollution (often referred to as particulate matter), sulphur oxides etc. these are from industrial and vehicle exhaust. A particle becomes a pollutant, when it is unable to degrade itself either naturally or artificially after its prescribed use. It remains in the environment for a longer period of time causing a disturbance in the natural cycles as well as effecting the native organisms, the plants and land in which it is present. A stable ecosystem will always bring balance in the environment, i.e. they can keep a check on pollution. But the fast growth in the population is causing a disruption in the balance of the ecosystem, where it is no longer able to recover itself.

One of the major pollutant is plastic. During the past three decades, plastic materials have been increasingly used in food clothing, shelter, transportation, construction, medical, and recreation industries, as they are strong, light-weighted, and durable. Currently, the global plastic production has crossed 300 million tonnes, which depicts the enormous application of petrochemical sources (around 4% of total petrochemical feedstock) in packaging industries (EUBP, 2016). Till date, different crude oil-derived polymers have captured the world plastic market because of their easy availability, low cost, good mechanical and barrier properties, ease of handling and moulding ability. Despite these advantages, non-biodegradable nature of the petroleum-derived plastic films causes severe environmental pollution (Avella *et al.,* 2005; Rhim and Ng, 2007; Kyrikou and Briassoulis, 2007). The successful production and marketing of biodegradable plastics will help alleviate the problem of environmental pollution. In the past 10 years, several biodegradable plastics have been introduced into the market. However, none of them is efficiently biodegradable in landfills. For this reason, none of the products has gained widespread use (Anonymous, 1999). Degradation of polythene is a great challenge as the materials are increasingly used. A very general estimate of world wide plastic waste generation is annually about 57 million tons (Bollag *et al*., 2000).

The population is directly proportional to the immediate exhaustion of natural resources as well as increased amount of environmental pollution. This environmental pollution has a direct effect on the health of the population. Exposure to high levels of air pollution can cause a variety of adverse health outcomes. It increases the risk of respiratory infections, heart disease and lung cancer. Both short and long term exposure to air pollutants have been associated with health impacts. More severe impacts affect people who are already ill (WHO, 2019). The polythene is the most commonly found non-degradable solid waste that has been recently recognized as a major threat to marine life. The polythene could sometimes cause blockage in intestine of fish, birds and marine mammals (Spear *et al*., 1995, Secchi and Zarzur, 1999). Combustion of plastic materials can release harmful chemicals, which upon inhalation can cause breathing difficulty, respiratory problems, liver dysfunction, headache, nausea, diarrhoea etc. Recently a discovery was made on the presence of microplastics in human blood stream (Leslie *et al.,* 2022). We have exceeded the limits of using plastic and there are a lot of researches going on in the background to reduce its use as well as effective ways of degradation and replacing it with other biodegradable material.

Many researchers are now involved in finding a way to reduce pollution. Various research and developmental activities are employed to tackle the growing concern regarding the same. By using biodegradable material and renewable sources of energy can bring out a reduction in the overuse of natural resources. Recently algae have received a lot of attention as a new biomass source for the production of renewable energy. The main characteristics of algal biomass are, they have a high biomass yield per unit area and light, do not require agricultural land, fresh water is not essential and nutrients can be supplied by wastewater and CO_2 by combustion gas. Microalgae can provide several different types of renewable bio-fuels. These include methane produced by anaerobic digestion of the algal biomass (Spolaore *et al.,* 2006) biodiesel derived from micro algal oil (Thomas, 2006; Roessler *et al.,*1994; Banerjee *et al.,* 2002) and photobiologically produced bio-hydrogen (Gavrilescu and Chisti, 2005; Fedorov *et al.,* 2005). The researchers are underway on the extraction of bio ethanol from the algal biomass. Heavy metals are major pollutants in marine ecosystems, lakes and ground waters, originating from industrial effluents even if they are treated for contaminant reduction. Biosorption is an inexpensive and reliable method to remove, for example, cadmium and lead ions from aqueous solution using dry seaweed biomass as adsorbents (Vinoj Kumar and Kalad-haran, 2006). Therefore, seaweeds offer great opportunities for biosorption because their macroscopic structure provides a convenient basis for production of biosorbent particles suitable for applications as sorption material (Michalak *et al.,* 2016). Algal biofertilizer can replace the commercial chemical fertilizers on the agricultural land. From ancient time onwards algae are being used as a compost and mulch. They are mixed with various soil and sand for improved crop production. Since seaweeds contain a lot of bioactive compounds, which will supplement the growth requirements of the (Nabti *et al.,* 2017). Kelp or *Laminaria, Turbinaria*, *Anabaena*, *Spirulina* etc. are the algal species used as biofertilizers. The majority of wastewater produced on land by anthropogenic activities is discharged, with varying levels of treatment, into the aquatic environment which accumulate in coastal waters, causing pollution and disturbances in the balance of marine ecosystems. Recent research and commercial developments in seaweed cultivation demonstrate the potential of seaweeds to remediate nutrients and metals from land-based and coastal aquaculture, urban and agricultural runoff, and industrial (Neveux *et al.,* 2018).

A break through finding was an effective way to degrade plastic. It was experimentally been proved that microbes cause degradation of plastic materials up to 28.8% within a month. The strains of *Aspergillus glaucus*, *A. niger*, *Pseudomonas* sp. and *Moraxella* sp. are efficient in biodegradation (Kathiresan, 2003). There are lots of reports demonstrating the potential of plastic degrading microbes, but none of them found to have practical application, thus there is a strong need to screen efficient organisms and developing technologies capable of degrading plastic efficiently without affecting environment (Kale *et al*., 2015).

Among total plastic film production, half is being utilized for packaging of disposable items and out of that about 20% is used for packaging of food items (Rhim *et al.,* 2013). This enormous usage of petroleum-based disposable plastics is significantly generating municipal solid waste every year if not, disposed of in ocean. Therefore, plastic industries are being urged to have eco-friendly plastic production to meet the desired functional and environmental attributes such as compostability, non-toxicity, and good mechanical and barrier properties. The rate of degradation, however, depends on microorganisms, environmental factors (light, oxygen and temperature), and mode of disposal, viz. landfills, terrestrial and marine environments (Hopewell *et al.,* 2009). Hence, many researchers are experimenting on different nature based materials to develop biodegradable films with the functional properties for packaging of food articles. One such natural material is algae. Hundreds of algal strains have been investigated with regard to the production biopolymers, mostly polysaccharides (Vincenzini *et al*., 1990). Polysaccharide family that includes cellulose, chitosan, starch derivatives, pectin derivatives, seaweed extracts, and microbial fermentation gums is generally used to make edible coatings (Cazon *et al.,* 2017; Thakur and Thakur, 2014; Thakur *et al.,* 2016). Polysaccharide coatings act as the agents delaying moisture loss from the food products (Azeredo and Waldron, 2016). A high diversity and quantity of polysaccharides are remarkable in diverse marine algae. These carbohydrates constitute cell walls and some are storage compounds within the cell (Cian *et al.,* 2015; Mwalugha *et al.,* 2015). In general, the different polysaccharides of seaweeds have a chemical structure according to the corresponding taxonomic classification of the algae (Chojnacka *et al.,* 2012). Their high polysaccharide content implies a high level of soluble and insoluble fibers. The composition of chemical constituents changes with the seasons and according to different environmental conditions, such as temperature, salinity, light and nutrient availability (Anantharaman *et al.,* 2010; Khairy and El-Shafay, 2013). Several algal polysaccharides have demonstrated valuable biological and pharmacological activities and it's found that they have great potentials as therapeutics.

Red marine seaweeds (Rhodophyta) are the source of some promising biopolymers since they contain considerable amounts of the polysaccharide agar, which has a unique structure. In seaweeds, agar fulfils a function analogous to that of cellulose in terrestrial plants, although it differs because marine seaweeds require a more flexible structure to resist currents and wave motion (Stanley, 1995). Polysaccharides can be extracted from the cell matrix of seaweeds of the Gelidiaceae and Gracilariaceae families. Fresh seaweeds can be obtained from the wild (i.e, natural growing conditions) or can be cultivated in-situ. The largest global production of agar is extracted from *Gracilaria* (53%) and *Gelidium* (44%), with only a small quantity (3%) coming from other species (Marinho-Soriano and Bourret, 2003). Due to over-harvesting and high demand, as well as the delay in developing *Gelidium* aquaculture until recently, the extraction of agar has focused on the resource of *Gracilaria* (Melo, 1998). *Gracilaria* spp. are therefore an important agarophyte resource globally, with production totalling 3,752,000 tons, which accounts for approximately 13.7% of farmed aquatic plants in the world (Officer FAO, 2016). However, the quality of the agar from *Gracilaria* is lower than that from *Gelidium* (Armisen, 1995).

The genus *Gracilaria* belongs to the phylum Rhodophyta (or red algae), class Florideophyceae, order Gracilariales, and family Gracilariaceae. *Gracilaria* is the largest genus in Gracilariales encompassing 190 species and, together with Ceramium Roth (209 species; Ceramiales) and *Polysiphonia greville* (209 species; Ceramiales), encompass the major genera of Rhodophyta (Guiry and Guiry, 2018). This genus was described by Greville in 1830 (Greville, 1830). The members of Gracilariales are dark red to yellowish red in colour, they are bushy and their height ranges between 9–17 cm. They have a rigid, cartilage of 2-4 mm wide, tips of the segments are acute, sometimes they are with proliferations, and thallus is composed of 1-2 layered cortex and medulla of large cells at the centre (Jha *et al.,* 2009). Agar from *Gracilaria* is a food-grade agar utilized as thickening, stabilizing or gelling agent for the baking and confectionery industry in the production of desserts, such as pies, icings, and jelly candies (Nussinovitch, 1997). They are better known as raw material exploited for the extraction of agar and mainly harvested from wild stocks and used for preparation of food grade agar in India. There are some efforts being made to cultivate them on a large scale.

Gracilaria corticata (J. Agard) J. Agard, (Figure 1b)(19.2% of studies) is the most frequently studied species, followed by *G. edulis*, G. *salicornia, G. changii, G. cornea*, and *G. gracilis* (Torres *et al.,* 2019). *G. corticata* is a predominant red seaweed species found in coastal regions of Indian subcontinent, belonging to the family Gracilariacea. It possess several biomedical properties such as antibacterial, antiviral, antifungal, antiprotozoal, anti-inflammatory, anti-oxidant, cytotoxic, contraception, gastrointestinal, cardiovascular, hypoglycemia, anti-enzymes, spasmolytic and allelophatic effects (Almeida *et al.,* 2011). Bioplastic prepared from *G. corticata* through the hot extraction of agar from the red seaweed collected from Karachi coast has shown highest solubility and decomposition rate with lowest agar concentration (Asif *et al*., 2021).

The main purpose of food packaging is the protection of foods against oxidation and microbiological spoilage. It creates active food packaging, which can enhance the protection by adding active substances: antibacterial and anti-oxidants. The addition of antioxidants in the packaging material provides advantages over the direct addition of food, reducing the amount of active ingredient introduced directly into the food product (Wróblewska-Krepsztul *et al.,* 2018). Keeping this in view, the aim of the present work was to find an effective replacement for plastic packaging films with a biodegradable plastic prepared by using natural polysaccharides extracted from the algae *Gracilaria* corticata collected from Thikkodi beach, Calicut district, Kerala and incorporation/addition of silica nanoparticles extracted from rice husk as well as essential oil from lemongrass, to enhance its qualitative characteristics. The objectives of the present work was framed as

- Extraction of polysaccharide and characterisation of the polysaccharide from Gracilaria
- Extraction and characterisation of nanosilica from rice husk
- Preparation of biodegradable plastic
- Characterization of the bioplastic synthesized

Review of Literature

Nowadays, we live in a world indispensable with plastic and it has become a part of our life. The amount of plastic generated per year is really high that everywhere in the neighbourhood what we see is a dump yard with plastic. Since it's non-biodegradable in origin, once made it remains forever. The invention of Bakelite in the early 19th century, has lead to the mass production of plastic, the rapid growth of industrialization contributed to this fact. The cost effective nature of plastic lead to its extensive use as packaging material. Because of this voluminous usage, it now remains a threat to the soil, water, air and the organisms. An alternative way of reducing plastic is to replace it with a biodegradable material. Recent studies have show that algal biopolymers are a promising way to achieve this goal. The widespread usage of biodegradable materials, can reduce the amount of waste, lower the greenhouse gas emissions and ensure the sustainable use of environmental resources. The following are some of the recent works done in this field of algal biopolymers.

The application of nanocomposite films based on natural biopolymers for packaging was discussed in a study conducted by Jong-Whan Rhim and Perry, 2007. They stated that there is a huge potential for the natural polymer-based nanocomposites to enhance quality and safety of packaged foods by increasing barrier properties of packaging materials with antimicrobial activity.

The studies conducted by Wu *et al.,* (2011) on the green algae *Neochloris oleoabundans* was shown to be able to produce large quantities (up to 5 g/L) of high viscosity polymers with 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. They suggested that properties of the polymer could be utilized in a variety of fields including food, cosmetic, and oil industries. Madera-Santana *et al.,* in 2011 conducted a study on the physicochemical properties of biodegradable polyvinyl alcohol–agar films from the red algae *Hydropuntia cornea*. The results indicated that, *H. cornea* collected in rainy season showed good properties for applications in the biodegradable packaging industry.

In 2014, Banerjee *et al.,* studied about algal biomass harvesting through cationic cassia gum, which is a natural plant based biopolymer. Their work emphasized on the synthesis of cationic cassia (CCAS) by the insertion of quaternary amine groups onto the backbone of cassia (CAS) from N-3-Chloro-2-hydroxypropyl trimethyl ammonium chloride (CHPTAC) which was further characterized via FTIR, SEM, elemental analysis and intrinsic viscosity. The harvested biomass was intended to be utilized as biofuel.

An experiment on the production of new cellulose nanomaterial from red algae marine biomass *Gelidium elegans* was conducted by Chen *et al.,* in 2016. They were able to isolate nanocellulose from *Gelidium elegans* red algae and it possessed high thermal stability than raw algae biomass. Nanocellulose having an aspect ratio of 25 with 73% crystalline was obtained. An extensive study on algal biopolymers was conducted by Özçimen *et al.,* in 2017. The types of algal biopolymers and many different processes to produce commercial bio-based polymers from algal biomass were presented to give point of view for using sustainable and renewable sources In a review article the evolution of hydrophobic cell wall biopolymers from algae to angiosperms were discussed by Karl *et al.,* (2017).

A study on reusing of red algae waste for the production of Cellulose nanocrystals and its application in polymer nanocomposites was carried out by El Achaby *et al.,* (2018). They were successful in extracting cellulose nano crystals (CNC) from red algal waste and nanocomposites with high mechanical performance and good transparency were obtained. In a review done by Sujosh Nandi and Proshanta Guha (2018), provides detailed procedure of cellulose nanocrystal extraction in a stepwise manner and discusses the changes in film properties after reinforcement into different biopolymers. Their studies reveal a promising way to create nano-biocomposite films as an appropriate counterpart of non-biodegradable plastics. A study on the recent progress in biodegradable polymers and nanocomposites based packaging materials for sustainable environment was conducted by Wróblewska-Krepsztul *et al.,* in 2018. They have briefly summarized the different characteristic of biodegradable polymers used in food packaging applications. Makaremi *et al.,* (2019) has studied about alginate-pectin based biocomposites used for food packaging. They have developed active, healable, and safely dissolvable alginate-pectin based biocomposites that have potential applications in food packaging. Their study revealed that, the fabricated biodegradable multi-functional biocomposite films possess various imperative properties, making them ideal for utilization as packaging material.

A method of preparing biocompatible antimicrobial alginate polymer from aqueous solution of commercial sodium alginate and aqueous extract of *wakame* using aminoglycoside antibiotics was described by Kumar *et al.,* (2019). Their study also revealed that, the 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. Another review on the potential of microalgae and their biopolymers as structuring ingredients in food was published by Bernaerts *et al.,* in 2019. They stated that microalgae can be considered as one of the promising sources of functional food ingredients because of their attractive composition in terms of nutritional and health-beneficial components.

In 2020, Fatemeh Kalateh Seifari and Hamed Ahari studied about the nanoemulsion encapsulation introduced as a technique for improving antimicrobial properties, while minimizing the impact of antimicrobial agents on the foods organoleptic properties. They proposed that 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 in 2021, conducted a study on chitosan based edible films. They have discussed about the latest development works (over the last five years) aimed at using chitosan (CS) in the manufacture of edible films and coatings for food preservation. Particular attention was given to relevant findings in the field, together with the novel preparation protocols of such biodegradable packaging. They have also addressed the recent trends in new concepts of composite films and coatings using chitosan.

The red seaweed *Porphyra columbina* was used to develop a naturally activated edible biofilms with antioxidant properties by Cian *et al.,* (2014). The aim of this work was to study the physicochemical and antioxidant properties of phycobiliproteins-phycocolloids-based films, obtained from mixtures of two aqueous fractions extracted from *Porphyra columbina* red seaweed, one enriched in phycocolloids (PcF) and the other in phycobiliproteins (PF). Agar films possess several properties adequate for food packaging applications. However, their high cost-production and quality variations caused by physiological and environmental factors affecting wild seaweeds make them less attractive for industries. In 2015, Sousa A. M and Goncalves M P, experimented on strategies to improve the mechanical strength and water resistance of agar films for food packaging applications. They found that by using a cheaper agar (NA) and LBG amounts as high as 50–75% could significantly reduce the cost-production and improve the properties of agar films. Hence sustainably grown seaweeds can represent a continuous supply of reliable feedstock for the packaging industry.

Extraction of sulfated agar from *Gracilaria lemaneiformis* was done by Huijing Chen *et al.,* (2020) an eco-friendly extraction method which was explored to obtain high sulphate content agar and repair the deficiency of enzymatic extraction by taking full advantage of hydrogen peroxide. The results obtained from this study, could provide useful information for the development of sulphated polysaccharides in food, pharmaceutical, cosmetic, and biotechnological applications. In 2010, biodegradable Agar extraction was carried out by Sousa *et at.,* from *Gracilaria vermiculophylla*. The objectives of this work was the production of biodegradable agar films from *Gracilaria vermiculophylla*, collected in Ria de Aveiro, Portugal, and the study of the effect of glycerol, an hydrophilic plasticizer, on the properties of the films and on subsequent application in edible coating of fresh fruits and vegetables.

Effect of lignin on water vapour barrier, it's mechanical, and structural properties of agar/lignin composite films were studied by Shiv Shankar *et al.,* (2015). They prepared biodegradable composite films using two renewable resources based biopolymers, agar and lignin alkali. The lignin was used as a reinforcing material and agar as a biopolymer matrix. Their results suggested that agar or lignin films have a potential to be used as a UV barrier food packaging material for maintaining food safety and extending the shelf-life of the packaged food. Jong-Whan Rhim *et al.,* (2011) conducted a research on the preparation and characterization of agar/clay nanocomposite films and there effect of clay type. Among the agar/clay nanocomposite films tested, only agar/Cloisite 30B nanocomposite film showed a bacteriostatic function against Listeria monocytogenes.

An experiment on degradation of agar films in a humid tropical climate was conducted by Freile-Pelegrı´n *et al.,* in 2006. Agar films were subjected to natural weathering and thermal, mechanical, morphological and structural changes were tested. Accelerated weathering exposure of agar films showed their reduction in size and hence causing contraction that leads to formation of micro-fractures and embrittlement, and promotes microbial attack. The agar degradation process data reported will be important in further research on potential uses of agar as an environmentally friendly solution to the problem of biodegradable composite disposal. Films made of agar and potato starch were elaborated and tested for a potential use as food packaging by Ying Wu *et al.,* in 2009. It was concluded that, the addition of agar improved microstructure of starch film, and then meliorated mechanical properties and water vapour permeability at high moisture environment. It widened the application of potato starch film in food packaging films.

In 2013, Begoña Giménez *et al.,* researched on active biodegradable films based on agar and agar–fish gelatin were developed by the incorporation of green tea aqueous extract to the film forming solution. It was found that agar–gelatin films were less resistant and more deformable than agar films. Addition of green tea to the films negatively affected the mechanical properties, the presence of gelatin in the agar–green tea film hindered the release of phenolics. Also it was noted that, gelatin decreased the antioxidant activity of the films, but not the antimicrobial activity.

An experiment on tapioca starch based edible films was conducted by development of model for barrier and optical properties by Prakash Maran *et al.,* 2013. The influence of film composition (tapioca starch, glycerol, agar and span80) on the barrier and optical properties of the tapioca starch based edible films was evaluated. The results showed that, hydrophilic nature and plasticizing effect of glycerol increases the water vapour permeability, oxygen permeability, moisture content, solubility and swelling capacity of the films. In 2011, Huafeng Tian *et al.,* the microstructure and mechanical properties of the blend films were evaluated in relation to the agar/protein ratio as well as the processing methods. Their experimental results revealed that hydrogen bonding interactions existed between soy protein and agar. It was noted that with the increase of agar in the casting blend films, the crosslinking density increased and was responsible for the variations in tensile strength.

Antimicrobial and physical-mechanical properties of agar-based films incorporated with grapefruit seed extract was studied by Paulraj Kanmani and Jong-Whan Rhim in 2014. Their present study was aimed to develop natural biopolymer-based antimicrobial packaging films as an alternative for the synthetic packaging films. The results suggested that agar/grapefruit seed extract films have potential to be used in an active food packaging systems for maintaining food safety and extending the shelf-life of the packaged food. Ping Zhao *et al.,* in 2020 conducted a research on agar extraction and purification of R-phycoerythrin from *Gracilaria tenuistipitata*, and subsequent wastewater treatment by *Ulva prolifera*. This study investigated the large-scale use of the red macroalga *Gracilaria tenuistipitata* to extract partially purified R-phycoerythrin in combination with the sequential extraction of agar. This extraction and subsequently eco-friendly wastewater treatment method could have practical application in the agar industry.

A study based on the seaweed *Gracilariopsis longissima* was conducted by Marta Alexandra dos Santos Veríssimo Freitas (2017), concerning research with distinct objectives. It mainly focused on the assessment of *G. longissima* growth rates according to salinity and under controlled conditions and to establishes a nutritional profile for *G. longissima*, namely by defining seasonal variations of protein content and determining the fatty acids profile, as well as providing insights on seasonal variations of antioxidant and antimicrobial activity. Their results showed that the protein content and a fatty acid profile rich in arachidonic and palmitic acid, while presenting weak antioxidant activity. It was also found that *G. longissima* also holds a remarkable antibacterial activity against the bacteria tested, with no seasonal significant differences detected. Overall, it was confirmed that, *G. longissima* stands as a potential natural source of biologically active compounds, being thus theoretically relevant for a wide range of biotechnological applications. A review focusing on the distinctive algal components that are beneficial in biomedical applications were published by Tanima Bhattacharya *et al.,* (2021). It also focuses on the research techniques to enrich the macronutrients and micronutrients by altering growth conditions and susceptible nutritional factors.

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:

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

Nanosilica synthesis from Rice husk

Nanosilica is synthesized by sol-gel method from rice husk (Fig. 3). 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.

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. Commercially bought lemon grass oil was added to incorporate antioxidant property to the bioplastic at a concentration of 20% 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 (Fig. 4).

Characterization of developed bioplastic film

Mechanical properties

Tensile Strength (TS) and elongation at break (EAB %) were analyzed using Universal Testing machine (Zwick/Roell, GmbH & Co, D-890 79 ULM) with a load cell of 1 KN and a test speed of 50 mm/min. Rectangular shaped strips (6 cm × 1 cm) of bioplastic films were clamped to the grips 10 cm apart from each other. Three values of the film sample were recorded.

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 month 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 (%) = $[(\mathrm{B1} - \mathrm{B2}) \div \mathrm{B1}] \times 100$

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 = $\frac{Control - Test}{Control} \times 100$

Results

Collection and processing of Seaweed

Gracilaria corticata was collected from Thikkodi beach, in Calicut district in the south west coast of Kerala state, India. Around 7 kg fresh specimen of *Gracilaria corticata* (Fig. 1) was collected and after drying under normal room temperature 420 g of dried powdered specimen was obtained.

Extraction of Polysaccharides from seaweed

The amount of polysaccharides extracted from cold extraction of 50 g dried powder of *Gracilaria corticata* is 19.99 g. The percentage yield is 39.98%.

Physical properties

Determination of solubility

The lyophilized polysaccharide powder was having an ivory colouration and it was soluble in common polar solvents like water and ethanol.

Characterization of polysaccharide powder

X-ray diffraction (XRD) analysis of Polysaccharide powder

The patterns obtained through the XRD characterization of the lyophilized sample are shown in Figure 5. By using OriginLab software, three major peaks were identified (Figure 6) and the crystallinity was calculated as 55.38%.

Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra of polysaccharide showed vibrational peaks at 3362.42 cm^{-1} which indicates the presence of O-H stretching and the corresponding broad peak is around 3400 cm^{-1} due the hydroxyl groups of polysaccharides and hydrogen bonding. In 1000 cm^{-1} there is CH bending

(Fig. 7). 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.

In vitro antioxidant activity

Antioxidant activity of the sample polysaccharides powder is shown in the table 1. The strongest antioxidant activity was at a concentration of 300 µl with an inhibition rate of 10.7%.

Synthesis of Silica nanoparticles from rice husk

The thermal treatment of 120 grams of rice husk has given an yield of 17.3 g of rice husk ash (RHA). After the acid precipitation process the final yield of nanoparticles from RHA, constitutes to 16.88 g, which is 97.57%.

Characterization of silicananoparticles

X-ray diffraction (XRD) analysis

From the XRD analysis and using OriginLab software (Figure 8 and 9), 4 major peaks were observed. Analysing the peaks and using the equation shown in materials and methods, it was found that the extracted nanoparticles were 98.43% crystalline.

Preparation of biodegradable bioplastic

The ideal concentration of glycerol and nanosilica particles for the preparation of bioplastic was found to be 11% and 5% respectively w/w polysaccharide (1.5 g). Along with it, essential oil from lemongrass (*Cymbopogon citratus* Stapf) was added in a concentration of 0.67% of the weight the polysaccharide powder. The developed bioplastic is shown in the Figure 11.

Characterization of Bioplastic

Mechanical strength

Several parameters are usually analysed in order to know the quality of the resultant bioplastic film. The bioplastic prepared was subjected to analysis the mechanical properties which mainly involves tensile strength. It was measured in triplicates (Table 2). The average maximum force and stress that can be held by the bioplastic before breakage was found to be 1.23127 N and 2.11208 N/mm^2. The elasticity was 49.0638N/mm^2 at a force of 0.2 to 0.3N at a speed of 20mm/min (Table 2).

Solubility rate

Water dissolving ability of the bioplastic prepared was found to be 14.08% (Fig. 12).

Degradation rate

The degradation test of the bioplastic after buried in soil for a week was found to be 6.17% (Fig. 13)

Antioxidant analysis

Antioxidant activity of the bioplastic prepared is shown in the table 3. The strongest antioxidant activity was at a concentration of 300 µl with an inhibition rate of 82%.

Table 1 *In Vitro* antioxidant analysis of polysaccharide powder

Table 1: In vitro antioxidant analysis of Polysaccharide powder

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

Table 2 Mechanical properties: Tensile Strength

Name	Max. Force	Max. Stress	Max. Stroke	Max. Stroke Strain	Elastic	Break Force	Break Stress	Break Stroke	Break Stroke Strain
Parameter	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas	Calc. at Entire Areas	Force 0.2 - 0.3 N	Sensitivity: 2	Sensitivity: 2	Sensitivity: 2	Sensitivity: 2
Unit	N	N/mm2	mm	%	N/mm2	N	N/mm2	mm	%
D _1	1.49446	2.98891	2.64637	8.82122	92.6197	0.36003	0.72006	3.47633	11.5878
D _2	0.76381	0.95477	3.26303	10.8768	10.779	0.21655	0.27068	3.9697	13.2323
D _3	1.43553	2.39256	3.25637	10.8546	43.7927	0.11935	0.19892	4.50973	15.0324
Average	1.23127	2.11208	3.05526	10.1842	49.0638	0.23198	0.39655	3.98525	13.2842
Standard Deviation	0.4059	1.04567	0.35412	1.18043	41.1742	0.12108	0.28245	0.51688	1.72289
Range	0.73065	2.03414	0.61666	2.05558	81.8407	0.24068	0.52114	1.0334	3.4446

Table 3 In vitro antioxidant analysis of bioplastic sample

Volume of sample (µl)	OD at 518 nm	% of Inhibition
100	0.286	58.2
200	0.157	77
300	0.123	82

Figure:1A: *Gracilaria corticata* (J. Agard) J. Agard. Habit

Figure 1B: Individual Thallus

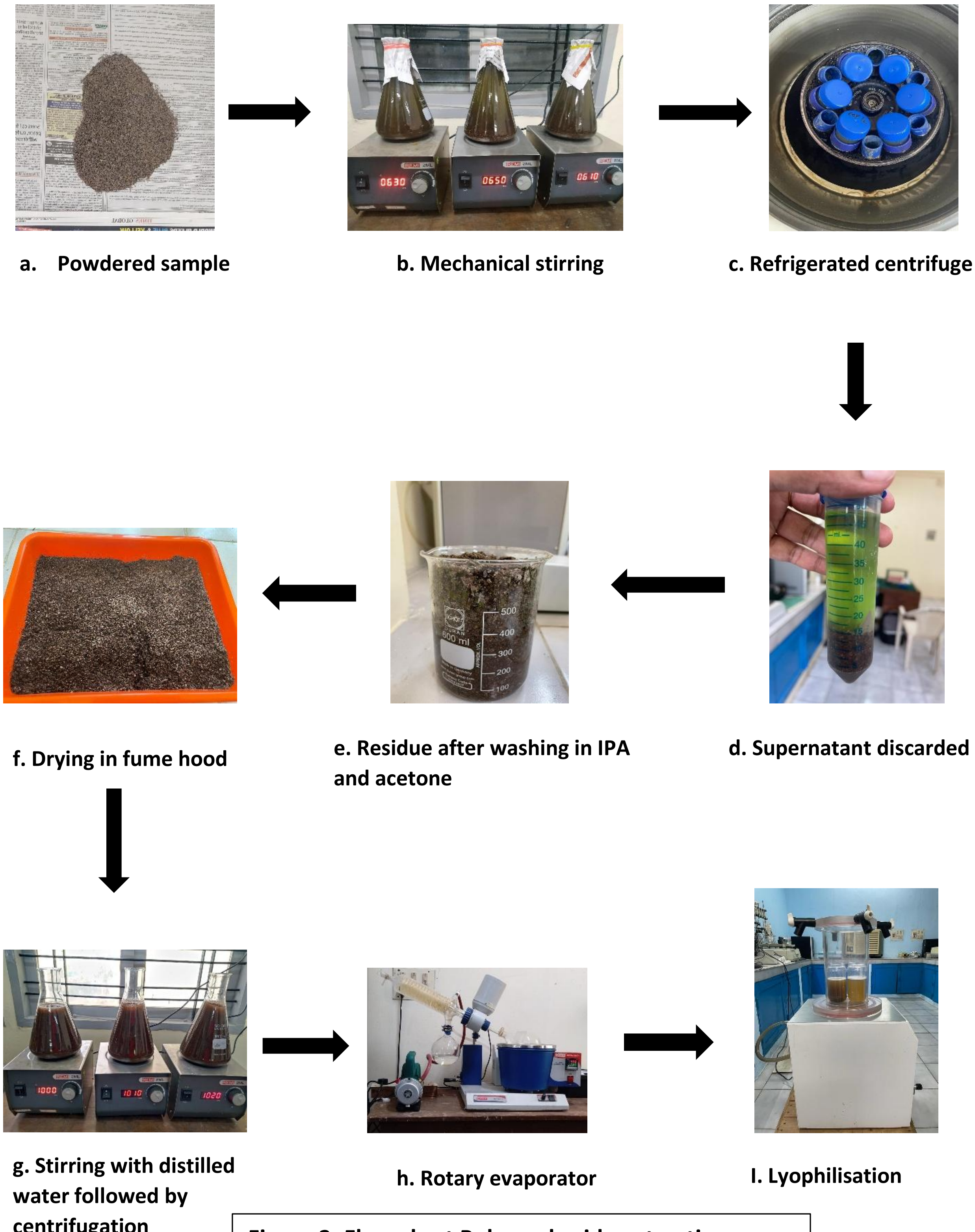

Figure 2: Flow chart Polysaccharide extraction

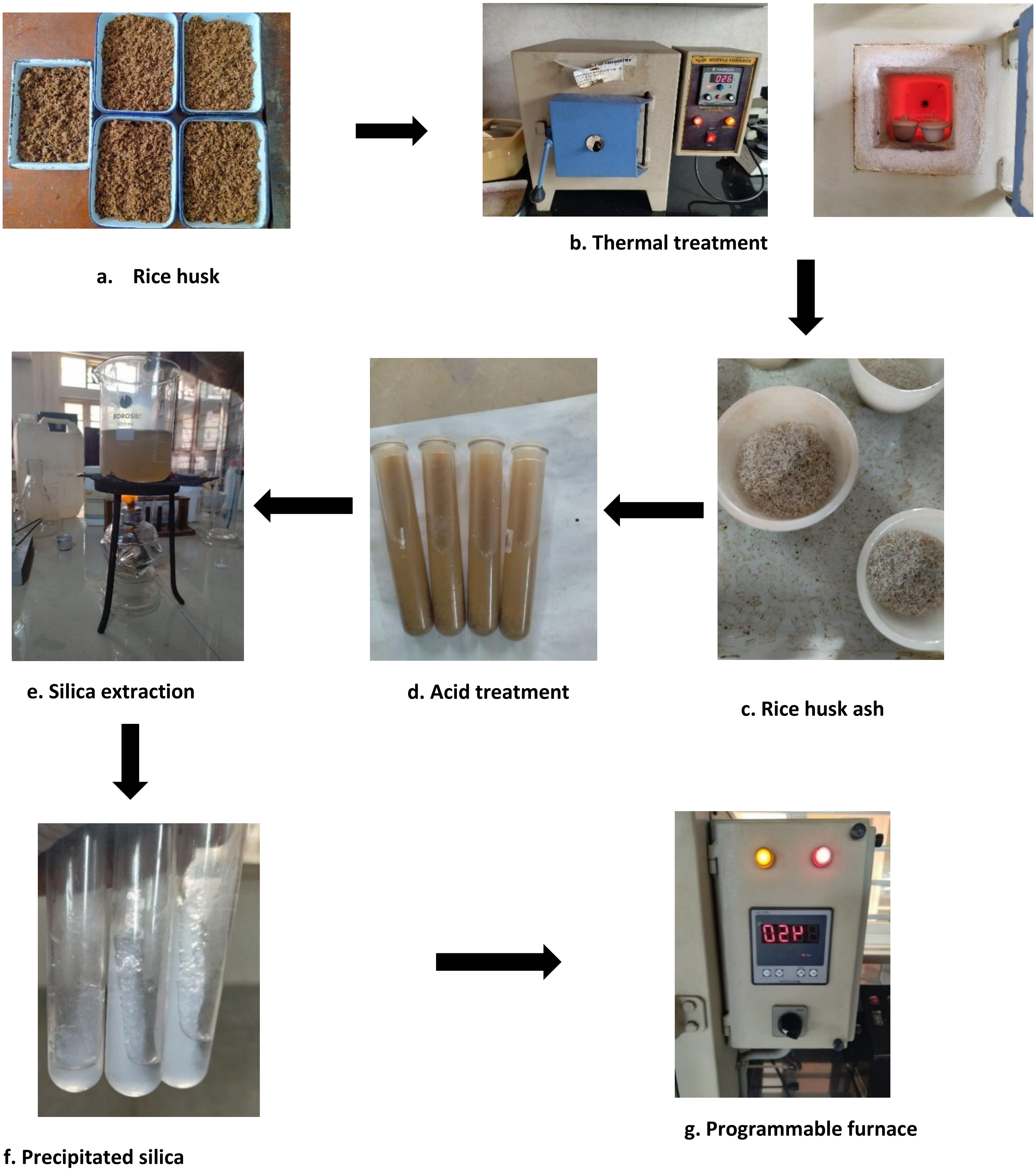

Figure 3: Flow chart Nanosilica synthesis

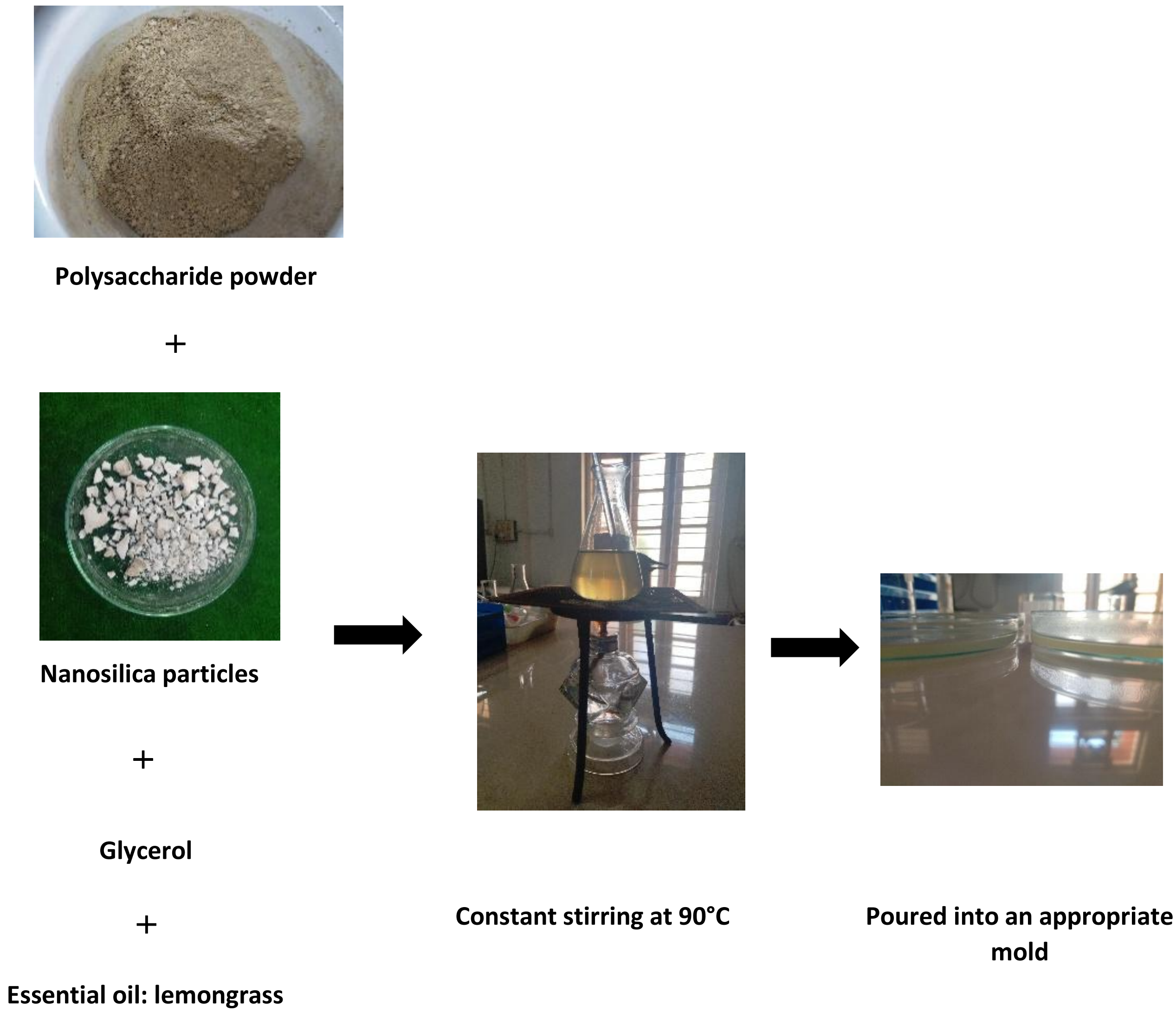

Figure 4: Flow chart Bioplastic preparation

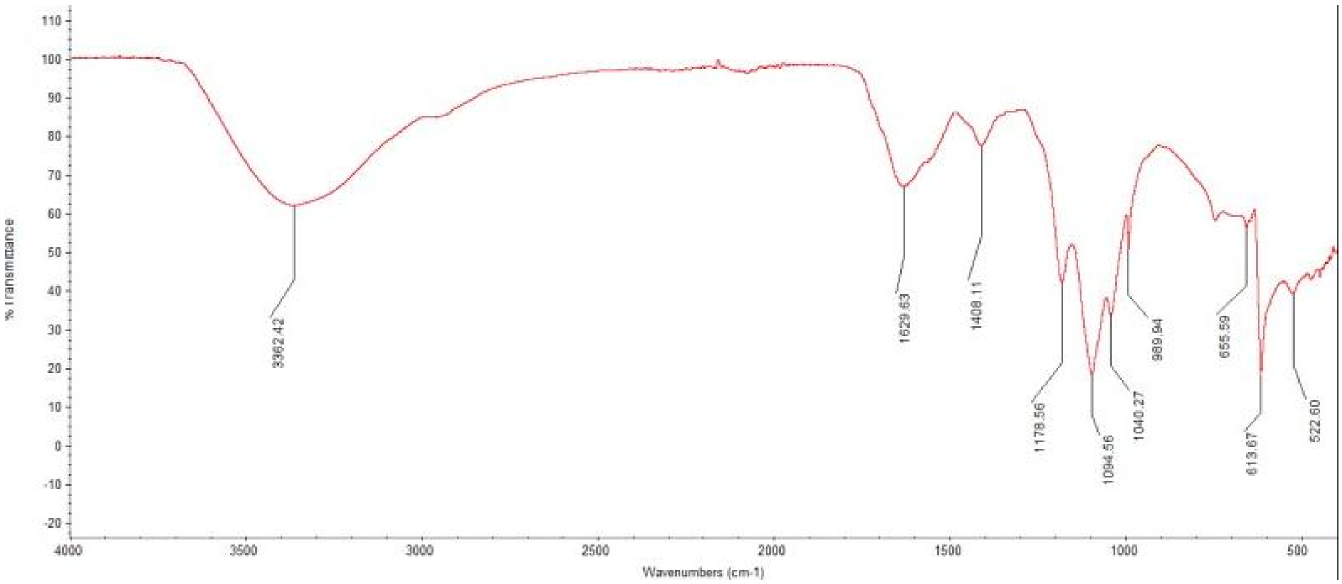

Figure 5: FTIR spectroscopy graph for Polysaccharide

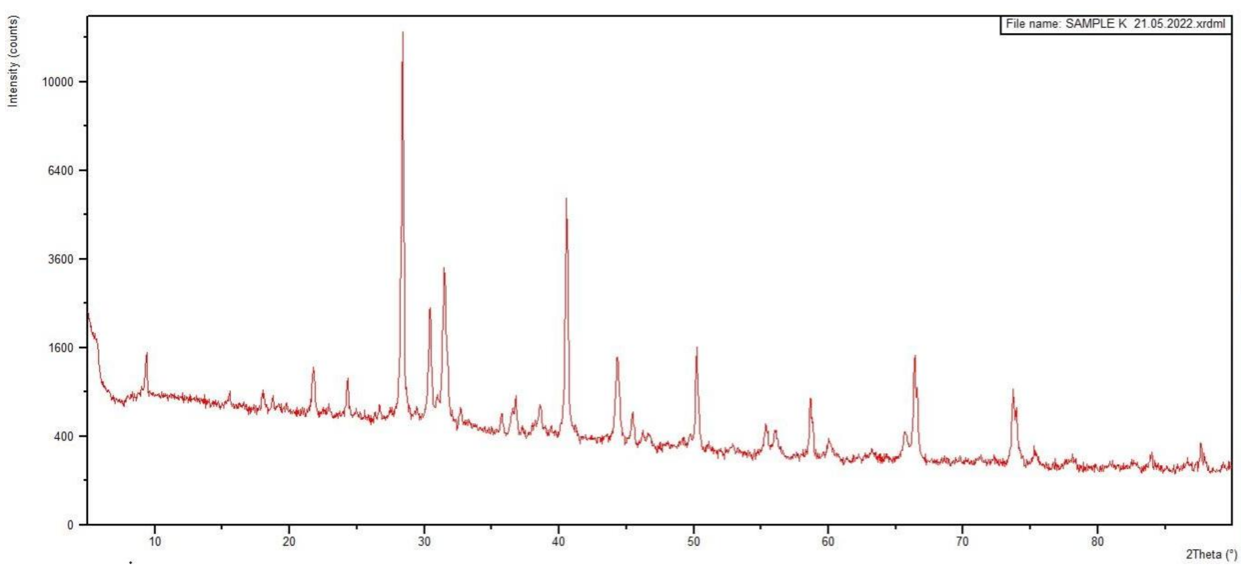

Figure 6: XRD spectrum of Polysaccharide powder

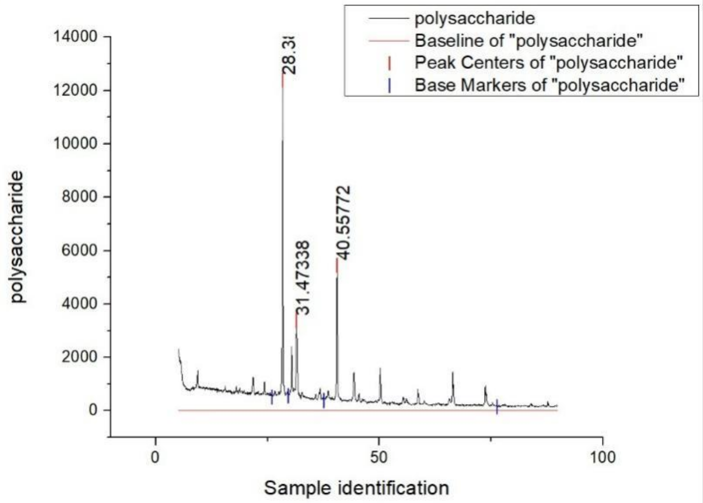

Figure 7 Peaks identified using OriginLab in Polysaccharide powder XRD data

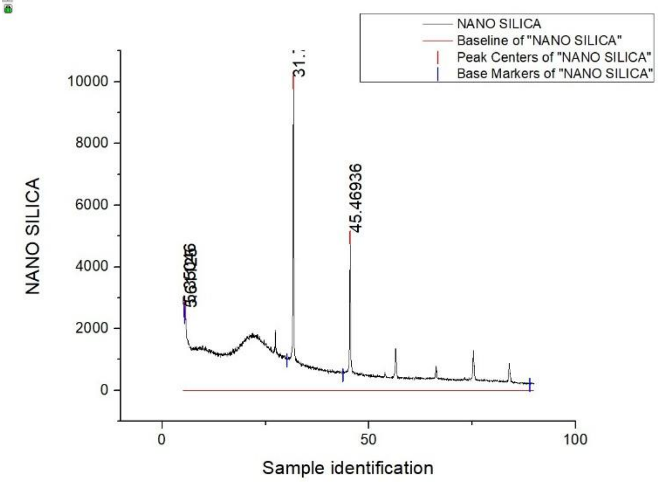

Figure 9 Major peaks identified using OriginLab in Nanosilica XRD data

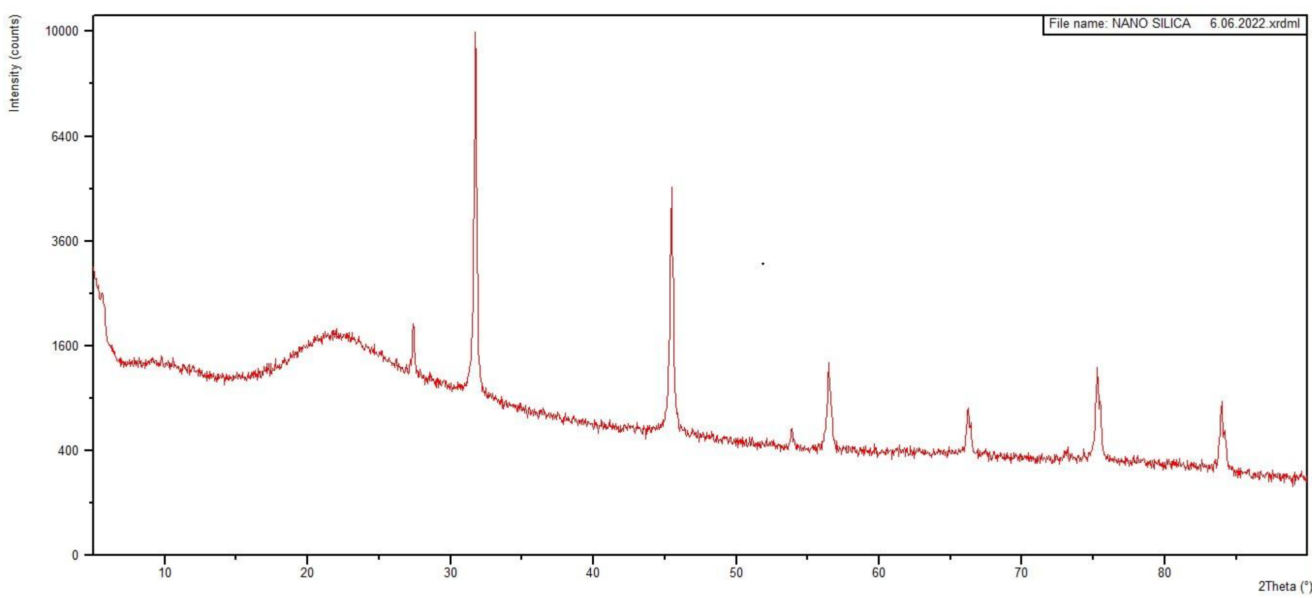

Figure 8: XRD spectrum of Nanosilica particles

Figure 10: Bioplastic in the mold

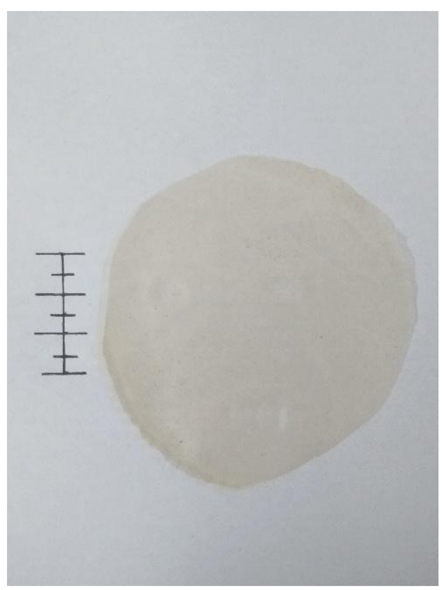

Figure 11: Bioplastic

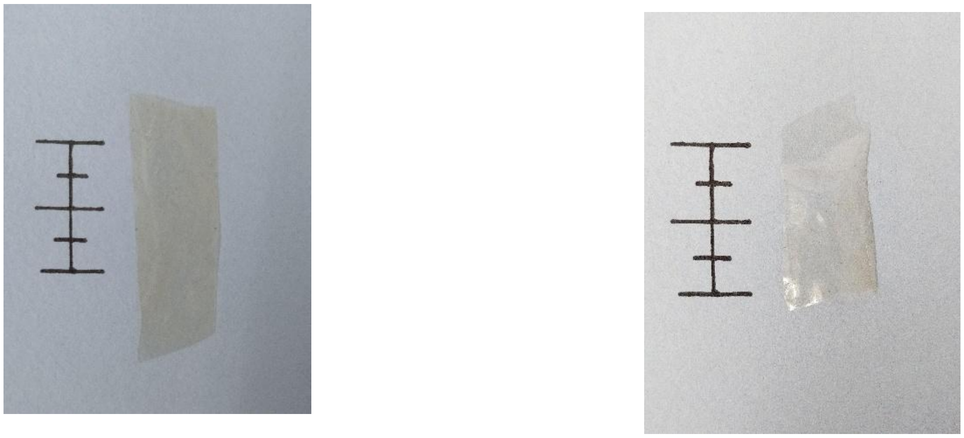

Figure 12: Solubility rate of the bioplastic; before (left) and after test(right)

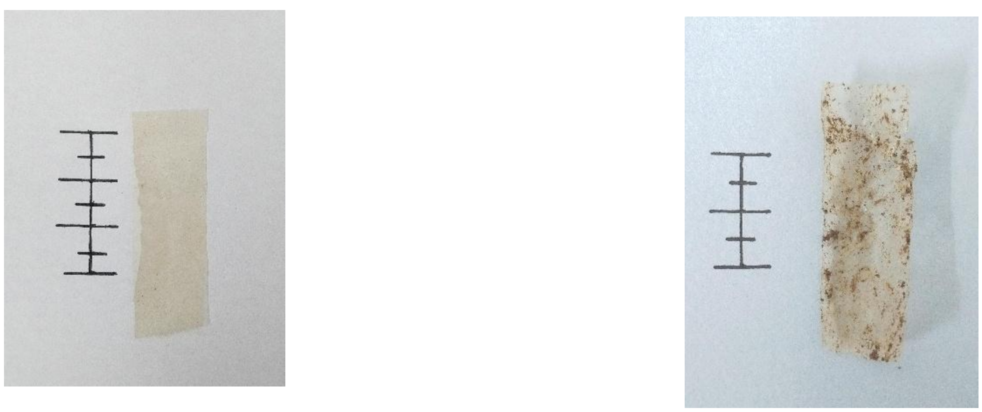

Figure 13: Degradation rate of the bioplastic: before (left) and after (right) test.

Discussion

The major part of the global marine biodiversity is represented by seaweeds. They form one of the commercially important marine living renewable resources. They are the only source for the production of phytochemicals such as agar, carrageenan and algin. They are especially dominant in the rocky intertidal zones of various coastlines. These rocky terrains provide a strong support for the attachment of the algae through the holdfast. The luxuriant growth of seaweeds is found in southeast of Tamil Nadu, Gujarat Coast, Lakshadweep and Andaman-Nicobar Islands. Rich seaweed beds occur at Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhituam, Pulicat Lake and Chilka Lake. About 220 genera and 740 species of marine algae have been reported so far from Indian waters. Thikkodi beach is a drive-in beach and one of the rising tourist attractions spot in the district of Kozhikode, Kerala. It is rocky in many parts and the salinity of the waters is high due to the absence of fresh water rivers in the nearby places. Thikkodi beach has more than 48 species of seaweeds and one among them is *Gracilaria corticata* (Palanisamy *et al.,* 2015). From the local information, luxuriant growth of seaweed in the beach was found to be in the months of December and January. The sample collection was done in the month of January and as expected a good yield of the sample was collected. In every coastal areas, there is an influence of high tides and low tides. During the low tides the seaweeds receives plenty of sunlight and its growth will be good. Approximately the low tides last for about 4 hours and it occurs twice during the day time in the study site, providing a good exposure of the sunlight and hence a better growth of algae. The growth and development of the seaweeds during the other seasons is not as evident as in the months of December to January to be noticed by the local community.

Currently, the most common polysaccharide extraction method is hot-water extraction and alcohol sedimentation, although the pharmacological activity may be decreased under the high extractive temperatures used (commonly 80–100 °C), which likely results in the degradation and oxidation of the polysaccharides (Chen *et al*., 2015). The identified seaweeds, *Gracilaria corticata* (Fig. 1) was processed for extraction of polysaccharides. The polysaccharides were extracted from *G. corticata* using cold extraction method (Fig. 2). Cold extraction instead of hot extraction was used in the extraction as the researchers found in their studies that cold extraction method gave higher yield of polysaccharides than hot extraction due to low extraction temperature. Research showed the extraction temperature is one of the factors which are responsible for the yield of extraction. The seaweeds were extracted under reflux using reflux apparatus as this technique prevents boiling dry of reaction vessel as vapour rapidly condensed in the condenser and the boiling can be proceeded at a constant temperature (Bhuyar *et al*., 2020). Besides, other extraction method of extraction such as methanol extraction, microwave and ultrasound extraction can also be carried out in order to identify the most suitable method in extracting high yield of polysaccharides from *Gracilaria corticata*. In the present study, the estimated total yield of polysaccharides was found to be slightly better than the hot water extraction method (Asif *et al.,* 2021). It was also noted that hot ethanolic extraction of *G. corticata* collected from west coast of Madagascan south island had considerable yield in comparison with the cold extraction protocol using isopropyl alcohol as solvent (Andriamanantoanina *et al.,* 2007). Research works of Villanueva *et al.,* 2010 (33% by *G. vermiculophylla*) and Wang *et al.,* 2017 (23% by *G. tenuistipitata*), showed better yield than the present study. The impact of cold techniques for the agar production is known to influence agar yield among seaweeds and may vary species to species (Soriano & Bourret, 2003; Nil *et al.,* 2016; Rasheed *et al.,* 2019). Yield and quality of agar can be modified or optimized by adjusting the pH while extraction (Kaliaperumal and Umamaheswara Rao, 1981; Rao and Kaladharan, 2003).

Polysaccharide extracted in the present study is water soluble hence it may contain galactose, mannose, polygalactose etc. The polysaccharides typically found in the genus *Gracilaria* are galactans of the agaran group. These compounds are constituted of residues of 3-linked β-D-galactopyranose (A units) alternating with 4-linked α-L-galactopyranose (B units) arranged in a linear backbone (Pomin & Mourão, 2008). The B unit is mainly 3,6-anhydro-α-L-galactopyranose residue. Pyruvic acid, sulfate, and methyl group are the main substituents in agaran. Agar is a complex mixture of different agarans composed of about 70% of agarose and 30% of agaropectin. Agarose is the neutral gelling fraction formed by the agarobiose, which presents alternating residues of D-galactopyranose and 3,6-anhydro-α-L-galactopyranose, while agaropectin is the non-gelling fraction formed by other agarans (Pandey *et al.,* 2015). FT-IR spectrum was taken for understanding the chemical bonding of extracted polysaccharides. FTIR spectrum showed various smaller and larger peaks. The absorption band in the spectrum is associated with methoxyl group, while vibrational band represents the CO and NH groups, which are responsible for the formation of conjugated peptide bonds (Elhefian *et al.,* 2012). The presence of bands at various points indicates the presence of anhydrogalactose bridges, which confirms the composition of extracted agar, which is in accordance with the previous reports (Pereira *et al.,* 2014). The spectrum obtained in the present study is similar to the earlier reports on the same species (Andriamanantoanina *et al.,* 2007; Rasheed *et al.,* 2019).

XRD technique is usually used for semiquantitative and qualitative assessment of amorphous and semicrystalline and crystalline component. Indeed, the crystalline or noncrystalline characteristics of a substance play a major role in the physicochemical properties by influencing the structural arrangements, like solubility, viscosity, and flexibility. The rays are diffracted in a pattern determined by the position, arrangement, and size of the constituents of the crystal. Scattered photons, which may undergo subsequent interference, lead to a characteristic diffraction pattern, which is specific to the crystalline powder and may serve as its 'fingerprint' (Tamiri & Zitrin, 2013). The XRD report of the present sample gives out a semi crystalline nature of the polysaccharide powder (figure 7). It is difficult to interpret broad amorphous peaks of several amorphous polysaccharide in X-ray scattering profile (Shimazu, Miyazaki, & Ikeda, 2000) while easy to interpret narrow crystalline peaks and therefore the ratio between sharp narrow diffraction peaks and broad peak was used to calculate the amount of crystallinity in the polysaccharide powder. According to the literature, this finding was similar to the results obtained by Ben Slima *et al.,* (2019), and these authors noticed that water-soluble polysaccharides extracted from *Sorghum bicolor* seeds were semicrystalline fibers. In the other study reported by Rashid *et al*., (2019), it showed that gum extracted from Katan seeds depicted amorphous behaviour (Hui, 2019).

Nanosilica particles are extensively used in the fields of ceramics, chemicals, catalysis, chromatography, energy, electronics, coatings, stabilisers, emulsifiers and biological sciences. For industrial applications enormous quantity of nanosilica powder is required. Nanosilica powder is generally prepared by using vapour-phase reaction, sol–gel and thermo-decomposition methods etc. (Kaviyarasu *et al.,* 2016). For the present study, a simple, convenient and economically viable method has been used to synthesise nanosilica crystals using sol-gel method which involves thermal, acid and alkaline treatments followed by acid precipitation method. The yield of nanosilica was found to be similar when compared with works which used slightly different pathways in developing the nanosilica crystals (Thuadaij and Nuntiya, 2008; Amutha *et al.,* 2010). The nature of nanosilica synthesized was characterized by x-Ray diffraction. It is easy to interpret narrow peaks than the broad amorphous peaks and hence it was found to be crystalline in nature. Also there is a broad peak (Fig. 9), suggesting the amorphous nature of the synthesized nanosilica particles. The XRD data of nanosilica synthesized from rice husk was amorphous (Thuadaij and Nuntiya, 2008; Amutha *et al.,* 2010 and Yuvakkumar *et al.,* 2014). Nanosilica that has amorphous structure can improve the strength of the concrete (Jal *et al.,* 2004). Nanoparticles often function as anti-microbial and reinforcing materials, when they are added to the polymers. So far in the field of packaging materials, industry has focused on layered clays and silicates, due to their availability, low cost and simplicity of adaptation. The interaction between silicates and polymer chains leads to perfect types of nanocomposites (Wróblewska-Krepsztul *et al.,* 2018). Hence by incorporation of silica nanoparticles for the preparation of biodegradable packaging plastic, will give a good durability to the end product.

The bioplastic was prepared from the mixture of extracted polysaccharide powder, synthesized silica nanoparticles, glycerol, and essential oil lemongrass in appropriate concentrations. The mixture prepared was poured into petri-plates coated with vegetable oil for smooth peeling of the bioplastic. The product obtained was opaque and had a light brownish colouration. It was slightly rough in texture and had minimal scent of the essential oil lemongrass. In recent years, various bioplastics, biofilm, biodegradable packaging films are being developed from natural materials and to improve its physicochemical properties various incorporation of starch, silica nanoparticles, silver nanoparticles etc., are seen. Tensile Strength is the maximum load large unity initial cross-sectional area of the sample. Tensile strength indicates the ability to accept load or tension without damaging the material in question or the point just before breakage when a maximum load is given to the material. Tensile strength of the bioplastic film can be affected by several factors, including the relative comparison between the matrix and the reinforcement materials in composite materials, namely how much nanosilica is added while preparing the bioplastic. The elasticity of the film is due to the addition of glycerol as plasticiser.

The materials used in the preparation of the bioplastic are all naturally obtained commodities and hence they are all individually biodegradable. In combination the results indicate that after one week of degradation there is a difference in the weight of the bioplastics. As the base material and plasticizers used in this study, glycerol are known to have hydrophilic properties (Vieira *et al.,* 2011; Hii *et al.,* 2016) therefore the sample showed weight loss. However the type of plasticizer can play a pronounced role in the decomposition test as shown by a comparison of sorbitol and glycerol as plasticiser incorporated in the bioplastics respectively prepared from the *Gracilaria corticata* by Asif *et al.,* 2021 in which glycerol incorporated bioplastic had highest degradation rate. This aspect could possibly be explained by the maximum moisture retaining by glycerol from the surrounding habitat, enabling the microbial growth. The more the water activity, higher would be the growth of microorganisms which can accelerate the degradation of samples (Hii *et al.,* 2016; Wahyuningtiyas & Suryanto, 2017). The rate of solubility of bioplastics is an important feature reflecting the ability of bioplastic being disintegrated in the presence of moisture, post-consumption, when utilized commercially (Arham *et al.,* 2016). The solubility rate of the prepared bioplastic was found to be lesser in comparison with the bioplastic prepared from the same species *Gracilaria corticata* collected from Karachi coast (Asif *et al.,* 2021). Bioplastics which has a low grade solubility are considered to be the best as they resist moisture for a longer period of time as they helps to increase the shelf life of a products; whereas, some edible bioplastics used in the packaging of food material mostly degrade rapidly (Sanyang *et al.,* 2015; Arham *et al.,* 2016; Giyatmi *et al.,* 2017). The bioplastic film to used as a commercial packaging film, it must posses certain qualities. For this reason, the mechanical properties may be considered as the most important of all the physical properties of bioplastic for most applications along with the solubility rate.

Oxidation is a normal process in the body. As a chemical reaction it produces byproduct called free radicals. A free radical is an oxygen molecule that has lost an electron and is in an unstable state, it tries to obtain an electron from other molecules and that leads to chain reactions which damage the cells of the organism. Antioxidants are the compounds that can inhibit oxidation and normally there is a balance between oxidation and antioxidants in the body. Naturally our body produces powerful antioxidants like alpha lipoic acid and glutathione and the rest of the antioxidant need is met from food we eat like fruits, vegetables, eggs and nuts. Over time these radicals build up and aging occurs. Normally free radicals can protect us from infection causing pathogens. But sometimes there occurs an imbalance between free radicals and antioxidant present in the body this condition is called oxidative stress. Overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, protiens, DNA), eventually leading to chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, post ischemic perfusion injury, myocardiac infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in human (Bhuvaneswari *et al.,* 2013; Fang *et al.,* 2002). Hence antioxidants are an essential component in the diet to prevent oxidative damage of the cells and eventually to be free of these lifestyle diseases.

The antioxidant activity of the bioplastic was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. This method is widely used to test the ability of compounds to act as hydrogen donators or free radical scavengers, to evaluate the antioxidant capacity. DPPH (1, 1-diphenyl-2-picryl hydrazyl radical) is known to be a stable radical at room temperature, which accepts electron or hydrogen radical to become a stable diamagnetic molecule (Soare *et al.,* 1997). It has been used to determine the antioxidant activity of various neutral products (Hu *et al.,* 2000). The effect of antioxidants on 1,1-diphenyl-2-picryl hydrazyl (DPPH) had been thought to be due to their hydrogen donating ability (Sun *et al.,* 2002). The antioxidant assay of the bioplastic prepared in the present study has shown an increasing amount of radical scavenging ability with increase in concentration owing to the addition of lemon grass oil. *Cymbopogon citratus* commonly is an aromatic, perennial grass belonging to the family Gramineae (Negrelle & Gomes, 2007). It is a tropical plant, grown as an ornamental plant in many temperate areas with maximum height of about 1.8 m and its leaves 1.9 cm wide covered with a whitish bloom (Prins *et al.,* 2013). In certain medications, it is used for mental illness. It is an antifungal, antioxidant and deodorizing agent. In combination with other herbs, it has large use as cure for Malaria (Cheel *et al.,* 2005). One of the main constituents of the many different species of lemongrass (genus *Cymbopogon*) is citral (Ademuyiwa & Grace, 2015; Agbafor & Akubugwo *et al.,* 2007) . Lemongrass oil has been found to contain up to 75-85% citral (Balakrishnan *et al.,* 2014). Lemongrass also contains z-citral, borneol, estragole, methyleugenol, geranyl acetate, geraniol, beta-myrcene, limonenepiperitone, citronellal, citrat-2, alpha-terpineole, pinene, farnesol, proximadiol and cymbodiacetal (Prins *et al*., 2013).

The use of plastic packaging is always on rise, the increased demand is due to population growth and market expansion. However, there are also increasing concerns about the harm caused to the environment and human health. These concerns include littering and accumulation of nondegradable plastics in the environment, generation of secondary microplastics and nanoplastics, and release of hazardous chemicals during manufacturing and use, as well as following landfilling, incineration, or improper disposal leading to pollution of the environment (Groh *et al.,* 2019). Some polymers used in packaging, including PS, PVC, PC, and PU (the latter used often in adhesives), are regarded as highly hazardous, while polyolefins and PLA are considered to be of lower hazard (Rossi and Blake, 2014). However, uncompounded polymers are rarely used in final applications, as various additives are usually added to modify polymer properties. If hazardous, these substances can lend hazard properties to even a seemingly safe polymeric material. Indeed, it is observed that the majority of plastic packaging-associated substances identified as the most hazardous for environmental and human health are plastics additives (Groh *et al.,* 2019). Infact many packaging plastic films under the label chemical free are highly toxic. There toxicity can leach out into the food items and can cause adverse effects on the one consuming it. They can alter several body functions and pathways which can lead to variety of lifestyle diseases. Bio packaging films with neutral activity will be a good replacement for there toxic counterparts.

There are a lot of antioxidants available in the nature like fruits, vegetables essentials oils etc. Among essential oil, lemongrass oil has a significant antioxidant activity (Balakrishnan *et al.,* 2014). The incorporation of lemongrass oil into the bioplastic solution is found to have enhanced the chemical properties of the bioplastic synthesised. Essentially it has an added antioxidant property which can contribute to its edibility status. In the present study, the prepared bioplastic has a great potential for being developed as a commercial packaging film and they are a better alternative to toxic counterparts in the packaging fields, as plastic films are both, a health and environment hazard.

Summary and Conclusion

Plastics are widely used commodity especially as food packaging. In general, plastic raw materials are polymers which have advantages including good mechanical properties, cheap, lightweight and easy in the process of manufacture and application. However, there are still many plastic shortcomings, one of them is not easily biodegradable. Around the world there is great concern to overcome the accumulation of non-biodegradable plastics in the environment by develop plastic biodegradable. The aim of the present study revolves around the same.

In the present study, bioplastic was prepared from naturally occurring materials, with the main component as polysaccharide obtained from the marine seaweed *Gracilaria corticata* (J. Agard) J. Agard, from the Thikkodi beach of Kozhikode district Kerala. A modified process was used for extraction of polysaccharides from seaweed. This is one among the first study in which the Rhodophyte *Gracilaria corticata* collected from Thikkodi beach Kozhikode district has been used for the preparation of bioplastic. The various bonds formed, nature of the polysaccharide powder and the radical scavenging ability was evaluated using Fourier-transform infrared spectroscopy, x-ray diffraction and antioxidant analysis. The other main components used in the preparation of bioplastic were nanosilica particles synthesized from rice husk, glycerol and essential oil lemongrass.

Rice is one of the staple food crops in the world. The production rice crops generate an equally great amount of waste materials in the world, which is commonly known as rice husk. Nanosilica extraction from rice husk is an age old technique. A slightly altered method was used for the synthesis of nanosilica. The properties of silica nanoparticles was characterized using x-ray diffraction, which is a very powerful analytical technique widely used for phase identification of materials. Nanosilica being a useful product developed from a waste material, it will not contribute to the ecological footprint. To improve the polymer performance nanotechnology offers innovative solutions. The addition of nanoparticles has improved the tensile strength of the bioplastic.

Lemongrass (*Cymbopogon citratus* Stapf), is of great interest due to its commercially valuable essential oils and widely used in functional food as well as in traditional medicines. The incorporation of lemon grass oil has not only brought a specific scent to the bioplastic, but it has also inculcated its antioxidant properties to the product owing to its edibility status. It is evident that the addition of antioxidant agents will significantly increase the pharmacological properties of the product.

The main disadvantages of the different types of bioplastic produced, is its low mechanical properties, low heat resistance, easy solubility and biodegradability. The bioplastic produced during the present study has been found out to be having a high solubility rate, and considerable mechanical properties, which can contribute as a potential alternative to the toxic packaging materials prevailing in the food packaging industry.

Various conservative methods are executed for the protection of environment and preserving it for the future generations. One of the major concerns being pollution by plastic polymers which are being tackled by the industrial progress in packaging technology in developing bio based materials. Over the years educating the mass regarding the rising pollution and concerns about protection of the environment has lead to the development of new innovative ideas for the evolution of sustainable practices. Many young researchers are in the quest for finding a proper biodegradable bioplastic and the present study contributes to achieving this dream.

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