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Preparation and characterization of packaging film using Carrageenan from Red seaweed and Green seaweed (*Kappaphycus alvarezii* (Doty) Doty ex Silva)

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Abstract

The aim of this work was to produce carrageenan films which can be used as a beneficial packaging material. In order to produce a substance like carrageenan was extracted from red seaweed and green seaweed *(Kappaphycus alvarezii)* by the method of Sulfated polysaccharide and the acquired carrageenan was made into films with additives like gelatin, lemon oil. Plasticizer like glycerol was also added to obtain elongation, transparent and flexibility of the films and coating was performed on fruits to prevent them from quick shrinkage and damage. The film was checked for its thickness, solubility, transparency. The physical, chemical parameters were evaluated by performing the characterization studies like FTIR, SEM and tensile strength and elongation at break. The antioxidant, anticoagulant properties, antimicrobial and cytotoxicity were tested to analyze and evaluate the quality of the films and organoleptic test were performed to analyze the shelf-life of the films. The FTIR results proved that the film has functional groups of sulfate and other added components. And other results proved that the film was completely safe to be used as a food packaging material. Biopolymer based carrageenan films can be effectively used as food packaging material for fruits and vegetables, which is organic, nontoxic, biodegradable and eco-friendly in nature.

Seaweeds are also known as marine algae. Seaweed based degradable polymers are produced from renewable source and edible components such as, proteins, lipids and polysaccharides. This type of polymer have been used in biomaterial products or wrappings or as packaging films in food coatings due to its preservative capabilities and its degradable properties, which are great advantage to the health and environment. Most abundantly used in the production of film due to its excellent film characteristics. *Kappaphycus alvarezii* (Doty) Doty ex Silva is one of the major commercial sources of carrageenan. *Kappaphycus alvarezii* is an important carrageenophyte which is able to produce over 80% of world's

carrageenan. Seaweed-based biopolymer used to make biopolymer films has importance on highquality and long-shelf-life-products and their awareness of environmental issues. Petrochemical plastics like, polystyrene, polyethylene and polyvinyl chloride, contains toxic substances such as, styrene and benzene which causes serious problems on the environment and humans because of the material's inability to biodegrade. Biopolymer films will be alternative for synthetic films in food packaging applications, because it has environmental friendly materials.

Carrageenan is a family of linear sulfated polysaccharides that are extracted from seaweed[21]. Carrageenan has all ability to become good packaging materials, because of their capacity of preserving food efficiency. Kappa forms strong, rigid gel, it was produced mainly from *Kappaphycus alvarezii*. Carrageenan was the excellent choice for the making of films with great oxygen barrier and low hygroscopic properties. The importance of carrageenan based biopolymer films is to increase the shelf life of the product (fruits and vegetables) plasticizer used to improve hydrophobicity of the film. It helps to maintain preservatives and flavour enhancer that improves the quality of foodstuffs. For the preparation of the manuscript, the authors have consulted the relevant literature[1-31].

Sample collection :

Samples of both red seaweed and green seaweed *(Kappaphycus alvarezii*) were collected from Rameshwaram Sea and washed thoroughly with tap water to remove debris. Species were identified and it was pre-treated with distilled water kept it in a sterile container.

Extraction of carrageenan :

Carrageenan was extracted with slight modifications in the method done by Badrinathan *et al*.,[9].

Characteristic analysis of carrageenan :

Carbohydrate test: (Molisch's test):

The dried sample was dissolved in 1 ml of distilled water in a dry test tube, Then 2 drops of Molisch's reagent were added along with that 1 ml of conc. H_2SO_4 was added within the side of the tube, so that two distinct layers are formed. Appearance of purple color indicates the presence of carbohydrate.

Solubility test :

Solubility test were done because carrageenan was insoluble in ethanol; whereas soluble in water at a temperature of about 80°C, forming a viscous color solution.

Test for sulfate:

Sulfate content determination was done by protocol given in FAO JECFA[12].

Qualitative test:

Qualitative test mainly to check whether sample has an ability to form a gel. Type of the carrageenan can also be identified spontaneously. 0.2 g of sample was dissolved in 0.1 N NaOH (sodium hydroxide) and 1 ml of $CaCl_2$ (calcium chloride) was added. If the

gel is formed, it indicates the presence of carrageenan and its morphology indicates its type.

Preparation of the film :

Preparation of Carrageenan films were made with a slight modifications in the protocol given by Ili Balqis. *et. al.,*[14].

Characteristic analysis of film :

Water absorption and solubility test:

Red seaweed carrageenan film and green seaweed carrageenan film was done with the standard method called Equilibrium ASTM D570. Film were cut into 3cm × 5cm dimension and weighed in weighing balance machine. Initial dry films weight was noted. The films were immersed in the water for 4 hours. Weight difference of dry and wet films and also solubility of films were noted.

Film thickness and swelling percentage:

Film thickness and swelling percentage of the carrageenan films were determined using the protocol done by Akshaya Krishnamurthy and Pavithra Amritkumar[2].

Film transparency :

Transparency of the film samples was determined with minor modification, according to method determined by Mulyono *et al.*[18].

Fourier Transform Infrared Spectroscopy Analysis (FTIR):

FTIR spectra of the films were recorded using an infrared spectrometer FT/IR-4100, JASCO. The spectra were obtained in the maximum frequency range between 4000 and 400cm^{-1}. Data analysis of each film was performed using FTIR spectrum software.

Scanning Electron Microscope Analysis (SEM):

The surface morphological structure of each film was analyzed in GEOL JSM 6360A electron microscope.

Tensile strength and elongation at Break:

Tensile strength was analyzed with the standard method ASTM D-882. Films were conditioned in the temperature of 23°C and 50% of relative humidity, the test was done by the paired sample grip the top and bottom. Size of the film samples used for the test is 8 × 4 cm (80 mm × 40 mm). The values were measured, graph and results documented.

Application study of Films:

Antimicrobial activity: Antibacterial activity:

About 200µl of *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus aureus and Bacillus* spp. were inoculated into Muller Hinton agar plates using spread plate technique. Films were cut into small circles and carefully placed in centre of the petriplates with the help of sterile forceps. The plate was left in the incubator at 37°C and zone of inhibition checked after 24 hours.

Antifungal activity :

About 200µl of *Candidas* spp. and *Aspergillus* spp. were inoculated into Potato

dextrose agar plates using spread plate technique. Films were cut into small circles and carefully placed in centre of the petriplates with the help of sterile forceps. The plate was left in the incubator at 37°C and zone of inhibition checked after 24 hours.

In vitro antioxidant activity: 1, 1-diphenyl–2 picrylhydrazyl (dpph) radical scavenging:

The DPPH radical scavenging method was used to evaluate the antioxidant activity. The concentrations of the film sample required to scavenge DPPH showed a dose dependent response. The antioxidant activity of each sample was expressed in terms of IC50, and was calculated from the graph after plotting inhibition percentage against film concentration DPPH assay was carried out after making some modifications in the standard protocol. 1.5 ml of 0.1mM DPPH solution was mixed with small circular piece of film samples. The mixture was shaken vigorously and incubated at room temperature for 30 minutes in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517nm by a Spectrophotometer. The solution without any sample and with DPPH and methanol was used as control. The experiment was replicated in three independent assays. Ascorbic acid was used as positive controls. Inhibition of DPPH free radical in percentage was calculated by the formula:

Inhibition (%) = [(A control – A test) / A control] × 100 :

Anti -coagulation assay: Thrombrolytic activity:

The fresh goat blood was collect from butcher shop and allowed to clot in the room temperature. The clot blood was cut into small pieces and put inside the eppendorf tubes. The small circular pieces of film samples were added to the clot blood in eppendorf tubes and checked for clot lysis. Determination of pt (prothrombin time): Whole blood was drawn from healthy human volunteers without a history of contraceptives or anticoagulant therapy. Each drop of blood was placed in sterile tile for samples and control. Small piece of film samples were kept in each drop of blood except control. The clotting time was observed and noted.

Cytotoxicity activity (mtt assay):

Cell (1×10^5 / well) (VERO CELLS) were plated in 96 well plates and incubated at 37°C with 5% CO_2 condition for 24 hours. After the cell reaches the confluence, media was removed from the wells carefully without disturbing the cells by using PBS buffer and Trypsin. The small circular shape of the film samples were added and incubated for 24 hours. After incubation, the sample was removed from the well and washed with phosphate buffered saline (pH-7.4) or MEM without serum. 100µl / well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazoium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with ELISA reader using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells/ A570 of control cells × 100

Organoleptic :

Organoleptic test was carried out by sensory observation (eye) to know the shelf-life of the film samples. This test was performed by unwrapped two fruits and wrapped two fruits (red seaweed carrageenan film and green seaweed carrageenan film). It was determined after three days of storage. It will evaluate the suitability, shrinkage of the fruit, and also observe any damage occur to the fruit.

Sample collection:

Samples were collected from Rameshwaram Sea water washed thoroughly with tap water to remove sands, impurities and foreign particles. It was again pre-treated with distilled water stored in the sterile container for extraction process (Fig. 1 and 2).

Extraction of Carrageenan:

The pre-treated sample of red seaweed and green seaweed was ground using sharp blade mixer in the laboratory.

↓

Ground sample (50g) of red seaweed and green seaweed were soaked in acetone for overnight.

↓

The residue of both red seaweed and green seaweed was filtered using muslin cloth and incubated 24 hrs, with 5 volumes of 0.25N of NaCl and 50mg of Trypsin.

↓

Incubated sample (250 ml) of both red seaweed and green seaweed was filtered using muslin cloth, the filtrate was precipitated using equal volume of ice-cold acetone and kept in deep freezer for 20 mins, more coagulation was occurred in red seaweed. When compared to green seaweed.

↓

The precipitate of red seaweed and green seaweed was centrifuged at 5000rpm for 25 mins, more pellets were settled at the bottom of the centrifuge tube in the red seaweed sample, whereas less amount of pellets were settled in the green seaweed sample, both were collected using glass rod, kept at room temperature for dying.

↓

Sufficient amount of dried carrageenan was occurred from red seaweed, whereas only small amount of dried carrageenan was occurred from green seaweed, both carrageenan samples were stored in the sterile container. Carrageenan was extracted by Sulfated polysaccharide method from both red seaweed and green seaweed (Kappaphycus alvarezii). Precipitated coagulum was observed to be more in red seaweed compared to green seaweed. So, the quantity of carrageenan from red seaweed was more than the green seaweed. The extraction resulted in brown solid substance. Carrageenan from both red seaweed and green seaweed has same color and texture. Same kind of extraction method was reported by Badrinathan. *et al.*,[9] from the species of brown seaweed *Sargassum myriocystum*.

Characteristic analysis of carrageenan

Carbohydrate test: (Molisch's test):

As in (Fig-3) Presence of carbohydrate in the samples was, confirmed by the appearance of purple color ring in the test tube. Similar carbohydrate test was done by Badrinathan. *et. al.*[9] with carrageenan from *Sargassum myriocystum*.

Solubility test :

As in (Fig-4) Samples were solubilized in water of about 80°C within 3-5 minutes and appearance of viscous solution indicated the presence of carrageenan. Similar kind of solubility test was performed in the research article of FAO JECFA[12] from different red seaweed species.

Test for Sulfate :

As in (Fig-5) Presence of sulfate content in the samples was, confirmed by the appearance of white crystalline precipitate at the bottom of the test tube, which indicated the characteristic of carrageenan. Similar kind of sulfate content test was performed in the research article of FAO JECFA[12] from different species of red seaweeds.

Qualitative test :

As in (Fig-6) carrageenan was proved as positive results from both samples by the formation of a strong, rigid gel and with its morphology it confirmed as Kappa carrageenan. Same kind of work was done with carrageenan from red seaweed by Saiful *et al.*, [23].

Preparation of the film :

Different concentrations of extracted carrageenan, gelatin, and lemon essential oil, glycerol, was tried to obtain a good quality films (Table 1).

Table 1. Standardization of carrageenan and other additive

Sample	Gelatin	Glycerol	Lemon Oil	Result
0.2 g	0.3 g	Few drops	Few drops	Very difficult to cast, very thin and Very difficult to peel.
0.3 g	0.5 g	0.1 ml	0.1ml	Very thin, difficult to peel, Sticky one.
0.4 g	1.0 g	0.2 ml	0.2 ml	Easy to cast, easy to peel with good consistency.

Fig-1: Red Seaweed (Kappaphycus alvarezii)

Fig-2: Green Seaweed (Kappaphycus alvarezii)

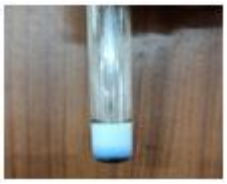


Fig-3: Molisch's Test

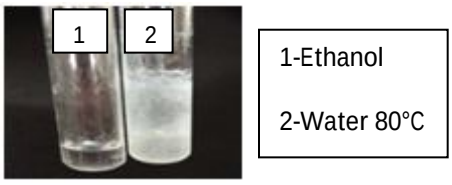
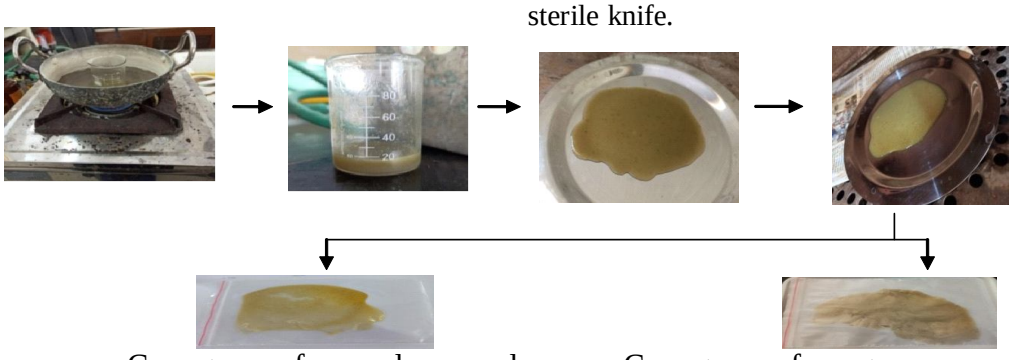


Fig-4: Solubility Test

Fig-5: Sulfate Test

Fig-6: Qualitative Test

As in (Fig-7) Standardized composition of developed film was obtained by dissolving 0.4g of carrageenan and 1.0g of gelatin in distilled water, stirred continuously at 80°C to dissolve completely. Glycerol of 0.2 ml was then added as a plasticizer and again stirred for few minutes and lemon essential oil 0.2 ml was added. Mixture was allowed to cool for few minutes and poured in the clean sterile steel plate, then placed in the hot air oven for 5 hrs. And kept aside, overnight at room temperature, next day peeled carefully using sterile knife.



Carrageenan from red seaweed Carrageenan from green seaweed

Fig-7: Procedure for developed carrageenan films

The carrageenan film from red seaweed was thick and easy to peel, whereas, carrageenan film from green seaweed was observed to be thin and little difficult to peel, when compared to carrageenan film from red seaweed and both films were stored in zip lock cover for further analysis. Films were prepared by casting method. Similar kind of kappa-carrageenan film from Eucheuma cottonii was reported by Ili Balqis. *et. al.,*[14] with different types of plasticizers at different concentrations.

Characteristic analysis of film:

Water absorption and solubility test:

Test results proved that the solubility of both carrageenan films has very high solubility, the whole films dissolves in 2 hours. Because of their higher solubility, absorption of water could not be determined. Hydrophilic nature was reason for the solubility of the films. Film of hydrocolloids (carrageenan) has very low resistance to water vapor but has good resistance to gases Oxygen (O_2) and Carbon dioxide (CO_2).Work similar to this have been reported using carrageenan film from red seaweed[23].

Film thickness and swelling percentage:

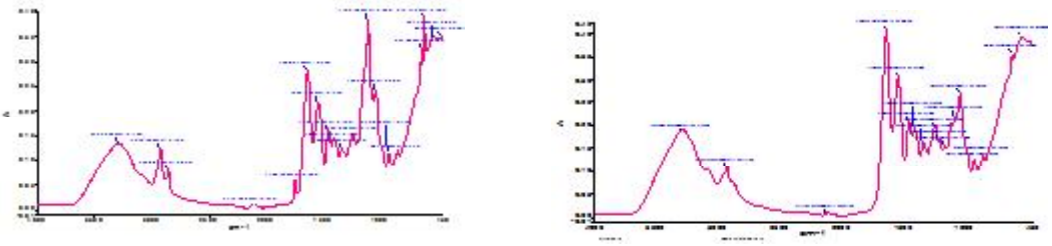
Table-2. Thickness and swelling percentage of films

Film type	Thickness before immersion (mm)	Thickness after immersion (mm)	Percentage
Carrageenan film from red seaweed	0.18	0.20	11.1
Carrageenan film from green seaweed	0.14	0.15	**7.**14

Film transparency:

Table-3. Transparency analysis of films

Film type	Absorbance	Transmission%	Thickness	Transparency
Cellophane cover (control)	0.398	39.99	0.12	13.34
Carrageenan film from red seaweed	0.337	46.02	0.18	9.23
Carrageenan film from green seaweed	0.233	58.47	0.14	12.62



(A) (B)

Fig-8: FTIR spectra of films (A) Carrageenan film from red seaweed (B) Carrageenan film from green seaweed.

The thickness and swelling percentage of two films was recorded (Table-2). Carrageenan film from red seaweed observed more water content than Carrageenan film from green seaweed, which could be due to the thickness of the films and moisture content, hence a higher swelling was observed in carrageenan film from red seaweed. When compared to other research studies, (Akshaya Krishnamurthy and Pavithra Amritkumar[28]) reported highest swelling percentage of 39.3 for PV film, whereas the film produced in our study gave 11.1 as a highest value, it was due to thickness of the film produced.

Film transparency :

Films showed lower transparency than cellophane cover (control) in (Table-3), which could be due to thickness of the films. Carrageenan film from green seaweed had good transparency when compared to carrageenan film from red seaweed. When compared to other studies, Carried out by (Akshaya Krishnamurthy and Pavithra Amritkumar[2]) achieved maximum transparency value of 5.86 for PAV film, whereas the films produced in our study had better transparency (12.62).

Fourier Transform Infrared Spectroscopy Analysis (FTIR):

The results of FTIR analysis of Carrageenan film from red seaweed and Carrageenan film from green seaweed (Fig-8) shows different stretches of bonds at different peaks which was used to know the possible chemical interactions in the films. Film (A) and (B) had more or less similar peaks in which the first peak at 3282.93cm^{-1} (A) and 3285.09cm^{-1} (B) indicated presence of carboxylic acid O–H stretch of strong bond, peak at 2925.35cm^{-1} (A) and 2922.82 cm^{-1} (B) showed alkane (C–H stretch) medium bond, peaks at 2854.88cm^{-1} and 2112.95cm^{-1} (A) and 2115.89cm^{-1}(B) indicated presence of C ≡ C stretching alkyne of weak bond, peak at 1745.85cm^{-1} (A) indicated cyclopentanone C= O stretching of strong bond, peak at 1634.57cm^{-1}(A) indicated medium bond of C= C stretch conjugated alkene, peaks at 1538.82cm^{-1}(A) and 1538.58cm^{-1}(B) indicated strong bond of nitro compound (N–O stretch), peak at 1458.26cm^{-1} (A) indicated C–H bending of alkane medium bond, peaks at 1239.48cm^{-1} (A) and 1239.53cm^{-1}(B) showed the presence of amine C–N stretch of medium bond, the peaks at 1403.71cm^{-1},1335.42cm^{-1} (A) and 1408.84cm^{-1} showed similar strong bond (S= O stretch) sulfate and sulfonate. Peak at 1079.39cm^{-1}(B) showed C–O stretching primary alcohol of strong bond, peaks at 1036.88cm^{-1}(A) and 1035.35cm^{-1} (B) indicated (S= O stretch) sulfoxide of strong bond, peak at 1335.76cm^{-1}(B) showed strong bond of C–N stretch of aromatic amine, peak at 1202.06cm^{-1}(B) indicated C–O stretch of vinyl ether strong bond, the peaks at 612.77 cm^{-1}, 513.16cm^{-1}, 637.45cm^{-1}(A) and 533.74 cm^{-1}, 614.93cm^{-1} (B) showed halo compound (C–Br stretch) strong bond, peak at 847.88 cm^{-1} showed the presences of strong bond of halo compound (C–CI stretch). The FTIR spectra of Carrageenan film of kappa/iota carrageenan from Mastocarpus stellatus showed similar range of peaks, in the research carried out by Blanco-Pascual. *et al.*,[10].

Scanning Electron microscope analysis (SEM):

The surface morphology of films was observed under SEM (Fig-9 and 10). Carrageenan film from red seaweed showed more white patches which indicated the presence of kappa-carrageenan in the film surface, whereas carrageenan film from green seaweed showed less white patches than carrageenan film from red seaweed. Interaction of kappa-carrageenan in the film was observed to be more in carrageenan film from red seaweed, whereas carrageenan film from green seaweed also has kappa-carrageenan interaction. Effect of plasticizer (glycerol) was also shown. White crystal like appearance was due to combination of gelatin in the films. Similar morphological surface of SEM images was observed in the k-carrageenan Blend films[15].

Tensile Strength and elongation at Break:

Tensile strength and Elongation at break in (Fig-11 A and B) showed the ability of films probity under stress and was important to determine their application. Variation in tensile strength and elongation at break was due to carrageenan and plasticizer content in the films. Elongation is a measure of films capacity for stretching. Tensile strength value of carrageenan film from red seaweed was 2.91 MPa, and elongation at break was 76.92% at the maximum load (N) of 7.28. Whereas, the tensile strength value of carrageenan film from green seaweed was 4.43 MPa and elongation at break was 61.52% at the maximum load (N) of 9.59. Similar kind of tensile strength and elongation at break results of starch/carrageenan blend films at different concentration were observed in the research carried out by other authors[1].

Application study of films:

Antibacterial activity:

Table-4. Antibacterial activity of Carrageenan film from red seaweed

Microorganisms	Film sample
Klebsiella spp.	7mm
Pseudomonas spp.	3mm
Staphylococcus aureus	3mm

Carrageenan film from red seaweed showed zone of inhibition against *Klebsiella* spp., *Pseudomonas* spp., *and Staphylococcus aureus* (Table 4). There was no zone of inhibition against *Bacillus* spp.

Table-5. Antibacterial activity of Carrageenan film from green seaweed

Microorganisms	Film sample
Pseudomonas spp.	3mm
Klebsiella spp.	2mm
Bacillus spp.	4mm

Carrageenan film from green seaweed showed zone of inhibition against against *Pseudomonas* spp., *Klebsiella* spp. *and Bacillus* spp (Table 5). There was no zone of inhibition against *Staphylococcus aureus*. The antimicrobial effect of Carrageenan film from red seaweed (Table 4) and Carrageenan film from green seaweed (Table 5) was evaluated against pathogens by agar diffusion method. Same kind of antibacterial activity of films prepared from carrageenan with different organisms was reported by El Fawal[11].

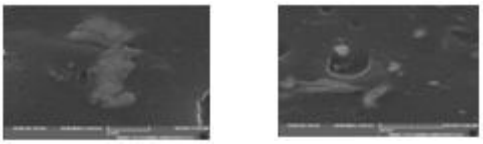


Fig-9. SEM images of Carrageenan film From red seaweed

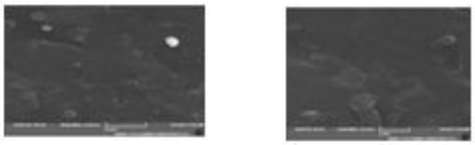
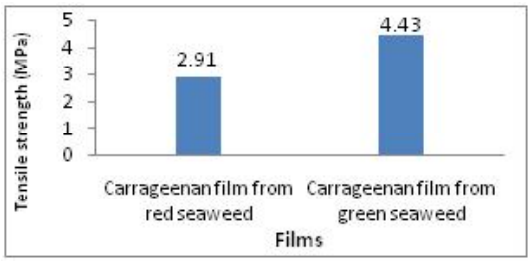
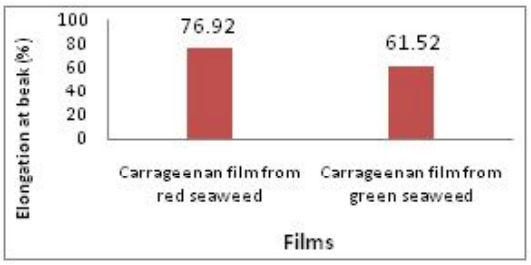


Fig-10. SEM images of Carrageenan film from green seaweed



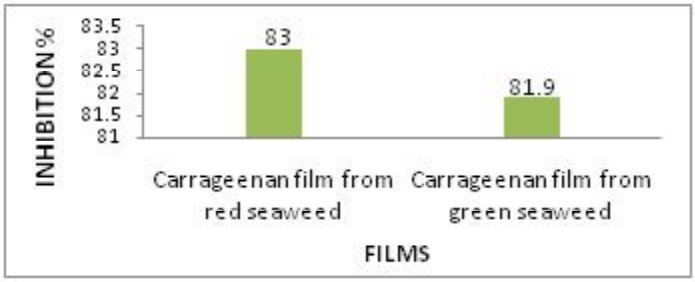
(A)



(B)

Fig-11 (A) Tensile strength of the films (B) Elongation at break of the films

(A)



(B)

Fig-12. antioxidant activity of films

1- Carrageenan film from red seaweed

2- Carrageenan film from green seaweed

Fig-13: Thrombrolytic activity of films

1- Control

2- Carrageenan film from red seaweed

3- Carrageenan film from green seaweed

Fig-14: Prothrombin activity of films

Antifungal activity :

There was no antifungal activity in Carrageenan film from red seaweed and Carrageenan film from green seaweed. However no antifungal activity was reported in any other carrageenan films research article carried out by other author El Fawal[11].

In Vitro Antioxidant Activity: 1,1-diphenyl–2 picryl hydrazyl (dpph) Radical Scavenging:

As in (Fig-12 A and B) the carrageenan film from both red seaweed and green seaweed showed good anti-oxidant activity. The anti-oxidant activity for carrageenan film from red seaweed was 83% and Carrageenan film from green seaweed was found to be 81.9%. The radical scavenging activity of carrageenan film from red seaweed was more than the carrageenan film from green seaweed. Antioxidant activity of the films can improve the preservative function and reduce the undesirable effects of nutrients oxidation. Work similar to this from k-carrageenan films containing essential oil was also provided good anti-oxidant property by other authors[25].

Anti coagulation asaay:

The thrombrolytic activity was said to be lysis of blood clot under invitro conditions (Fig-13). The carrageenan film from green seaweed and carrageenan film from red seaweed does not expressed, any lysis activity. Other research work with carrageenan sample from *Chondrus crispus*, carried out by Anderson. *et. al*.[4] have good thrombrolytic activity, due to the presence of sulfate content, whereas carrageenan film from green seaweed and carrageenan film from red seaweed in our study does not show any thrombrolytic activity

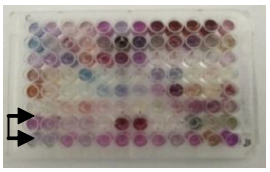


Fig-15: Cytotoxicity activity of films using MTT assay

Fig-16: Carrageenan film from red seaweed (Day 1)

Fig-17: Carrageenan film green seaweed (Day 1)

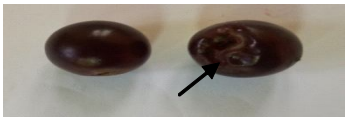


Fig-18: Carrageenan film from red seaweed (Day 3)

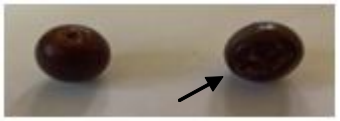


Fig-19: Carrageenan film from green seaweed (Day 3)

may be because of the combination of other additives used in the films have been influenced.

As in (Fig-14) indicates clotting time of the samples to coagulate the blood in invitro condition. Clot time was observed in carrageenan film from red seaweed was 40 seconds. The clot time showed in carrageenan

film from green seaweed was 35 seconds. Clot was formed in both the film samples, hence it indicated that films does not contain anticoagulation activity, so humans can use it safely because it will not affect coagulation property of blood. Other research work with carrageenan sample from Chondrus crispus, carried out by Anderson *et. al*.[4] have good anticoagulant activity, due to the presence of more sulfate content**,** whereas films produced in our study does not show good prothrombin time, due to the effect of other additives used in our films.

Cytotoxicity Activity (MTT assay):

Cytotoxicity activity of carrageenan film from red seaweed and carrageenan film from green seaweed shown in (Fig-15) was performed using MTT assay. Both carrageenan films were showed non-toxic, which confirmed that the films can be safely used as a food packaging material. The carrageenan film from red seaweed has 98.96% of viable cells, whereas carrageenan film from green seaweed has 81.3% of viable cells. The proliferating cells give high values hence, it increases the cell viability. The carrageenan film from red seaweed has more viable cells than that of carrageenan film from green seaweed. Same kind of research work showed less-toxic with degraded carrageenan by other authors Anderson. *et. al.*[4].

Organoleptic:

The arrow (⟶) in the (Fig-18 and 19) showed the unwrapped fruit which was kept before 3 days Organoleptic test shown in (Fig-16 and 17) was performed by sensory observation. Both wrapped and unwrapped fruits of both carrageenan films were spoiled in 3 days has in (Fig-18 and 19) whereas, the spoilage rate of unwrapped fruit was more when compared to wrapped ones. Therefore films preserve the fruits from quick shrinkage and damage. Whereas, films produced in our study was observed to be low resistance to water vapor, however our films more effectively preserve and prevent hard skinned fruits and vegetables from microbes, however the microbial growth was difficult to control. Similar research work was done with apple slices showed good prevention against shrinkage of slices in wrapped film, by other authors[23].

The isolation of carrageenan from red seaweed and green seaweed (*Kappaphycus alvarezii*) since, carrageenan are excellent family of natural polymers and widely used in food industry because of its gel forming abilities. Natural polymer films will increase shelf life, quality, reduce microbial growth, damage and shrinkage and also maintain pigments, flavor and nutrients. Whereas, packaging of food using petrochemical plastics will cause environment pollution and harmful to mankind and it was also a non-biodegradable material. Whereas, carrageenan film produced in our study as low resistance to water vapor, so films are more effectively preserve and prevent hard skinned fruits and vegetables from microbial growth, shrinkage and damage. It can be concluded that carrageenan films can be effectively used as food packaging material for fruits and vegetables, however organic, nontoxic, biodegradable and eco-friendly in nature film was produced. It will be a good alternative for usage in the food packaging

industry. They can mainly used for hard skinned and less moisture content fruits and vegetables to increase their shelf life. All the characteristics analysis study of SEM, FTIR, Tensile strength and elongation at break, films transparency and antioxidant, antibacterial, anticoagulant and cytotoxicity indicates that the films is a suitable material for food packaging.

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