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Preparation of Environmentally Friendly Bioplastics from Marine Red Algae, Salt Horned Seaweed, And Seaweed

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Abstract: ***The present study aimed to prepare sustainable and eco - friendly bio - plastics of agar extracted from the red seaweeds - Gracilaria deblis, Gracilaria salicornia and Gracilaria edulis. The agar was extracted by Hot - water extraction method (HWE) and the agar yield percentage was determined. The agar yield was high for Gracilaria edulis – 32% than the other two species. Bioplastics were prepared from the agar obtained from the three different species of Gracilaria. Sorbitol, glycerol, and starch were used as plasticizer to increase the elasticity and flexibility of the bioplastic films. The water solubility percentage was calculated for the bioplastics and it is higher for Gracilaria salicornia (86%) when compared with the other two species and found to have low strength and bioplastic made from G. deblis has low water solubility and considered to be the best when compared with other bioplastics as they resist moisture for a longer period and help to increase the shelf life of products. The prepared bioplastic was subjected to biodegradation test.1g of each bioplastic was buried in soil and reweighed after one month and the biodegradation rate was determined. The weight loss percentage was higher for Gracilaria deblis (47.9%) when compared with Gracilaria edulis (21.8%) and Gracilaria salicornia (16.6%). The results showed that the solubility of Gracilaria salicornia was higher than Gracilaria deblis and Gracilaria edulis. This proves that agar extracted from marine macro algae Gracilaria deblis, Gracilaria salicornia and Gracilaria edulis can be used as starting material for the synthesis of eco - friendly bioplastic.***

Keywords: Gracilaria, Bioplastic, Red Seaweeds, Hot water Extraction of agar

1. Introduction

Plastics play an important role in many fields and has many applications in our daily life. Though the demand for plastic is increasing, many countries also oppose of using non degradable plastic as it mainly causes environmental pollution and harm the aquatic animals when enter the aquatic bodies.

It takes many years to get completely degraded and many synthetic plastics are also recalcitrant and pose many problems like release of dioxins when the plastics are burnt. Moreover, recycling of plastics is difficult because different kinds of plastic must be recycled by different processes and involves high cost as the value of material is low **(Sneha, 2012).** Therefore, to overcome the problems of synthetic plastics, Bioplastics are an alternative source as they are biodegradable and have same applications as synthetic plastics. and can be prepared from plants, animals, and microbes.

Algae are the autotrophic organisms ranging from unicellular to multicellular forms (**Swiatek, 2010**). Microalgae such as Spirulina drugs are used in the production of bioplastics, but cannot be easily harvested. Whereas macroalgae like sea weeds have more potential than other sources because of its high biomass, ability to grow in wide range of environments, cost effective, easily cultivated, and harvested in natural environment (**Rajendran et al., 2012**). Since Seaweeds are edible, they can be utilized in food packaging industries.

Bioplastics can be degraded by microorganisms present in the soil such as bacteria and fungi without any release of pollutants. Moreover, the use of renewable sources in their manufacturing plays a key role in maintaining environmental plastics such as reduced dependence on fossil fuels, non - toxic easier to recycle, require less energy to produce, renewable and eco - friendly.

Seaweeds are one of the most important living resources known for their richness in polysaccharides, vitamins, minerals, fibres, and some other bioactive compounds such as proteins, lipids, and polyphenols some species of brown and red seaweeds have also been used to extract hydrocolloids such as Alginates, (**Rajan, 2012)** Agar and Carrageenan.

The use of agar with compatible plasticizer increases the flexibility and extensibility of film by reducing the brittleness of polymers. The most used plasticizers are polyols in hydrocolloid film production.

Seaweeds are rich in polysaccharides and they are used in the fields of food technology, Biotechnology, Microbiology and even medicine but not yet in the plastic industry. They can be used to produce biodegradable and high - quality bioplastic as they are inexpensive to cultivate.

Gracilaria is genus of red algae (Rhodophyta) found in warm waters throughout the world and they also occur seasonally in temperate waters. Gracilaria are found in all oceans expect the Arctic. *Gracilaria* is mainly cultivated and harvested for agar production. The three major species are

Gracilaria salicornia, *Gracilaria deblis* and *Gracilaria edulis.*

Gracilaria salicornia is one of the most successful invasive algae on reef flats. It has spread over 5 kilometres. This species successfully competes with other macroalgae by forming large, intricate mats that cover the substrate and inhibit settlement of other algae. It is also a source of agar production.

Gracilaria deblis is an economically important red alga. The cultivation of *Gracilaria deblis* is possible in Indian waters using raft culture method with six harvest cycles per annum. A feasible cultivation method has been developed for sustainable production of biomass and an improved process have been developed for extraction of superior quality agar for *Gracilaria edulis* which may be useful for food, agriculture, and biological applications. It is also used for gel formation and is used as thickening or gelling agents.

In the preparation of such eco - friendly plastics, plasticizers play a significant role by improving the elasticity and ductility of plastics (**Bourtoom, 2008**). The objective of the study is to prepare bioplastic using red algae - *Gracilaria* species.

2. Materials and Methodology

2.1 Collection of samples:

Marine red algae – *Gracilaria salicornia*, *Gracilaria deblis* and *Gracilaria edulis* were collected from CSIR - CSMCRI (Central Salt and Marine chemicals Research institute).

2.2 Processing of Seaweed

Prior to the agar extraction, collected Seaweeds were thoroughly washed with distilled water and was shade dried for 3 to 4 days. After complete drying, algae were ground into small pieces to facilitate extraction process

2.3 Hot water extraction of agar from Seaweeds:

Agar was extracted via native hot - water extraction method (Tabassum et., al 2016) Dried and chopped seaweed material was immersed in hot boiling deionized water in the ratio of 1: 5 (w/v) and followed by boiling for 2 to 3 hours. The left behind hot emulsion was then sieved two to three times repeatedly using muslin cloth. The filtrate was cooled to form gel and kept in freezer overnight. The frozen agar strips were thawed to remove as much water as possible and were subsequently oven dried at 50°c for 24 hours.1.5 g of dried agar was obtained from 10g of dried seaweed material. The dried agar strips were then converted into powdery mass for bio - plastic preparation.

2.4 Determination of agar yield:

The total amount of extracted agar (yield percentage) was evaluated depending upon the dry weight of seaweed and dried amount of obtained agar.

$$\text{Yield (\%)} = \frac{Dry\ wt.of\ Agar}{Dry\ weight\ of\ seaweed} \times 100$$

2.5 Preparation of Bioplastic

Extracted agar was used as main ingredient in the preparation of bioplastic by casting method (Arham 2016). Moreover, comparisons were made based on plasticizers added.3 g of Agar was weighed and heated with 300 ml of distilled water at 80°C to gelatinize with continuous stirring at 150 rpm for 30 minutes.1 g of Starch; 150 ml of glycerol and 1.5 g of sorbitol (Plasticizers) were then mixed with the thickened solutions at 80 °C with constant stirring to prevent any clumping. When the mixture became gummy, it was then poured into casting plates and dried at room temperature. When the mixture was completely dried, films of plastic were removed gently.

2.6 Determination of Water Solubility ratio

The water solubility (WS) ratio was determined as per basic standard method (Wang 2015). Total of three specimens from each sample were oven dried at 104˚C for 24 hours and weighed (W1) properly. The dried pieces were then immersed in centrifuge tubes containing 30 ml distilled water, kept in water bath at 25ºC with slow shaking overnight. The solutions were filtered and the remnants on filter paper were dried at 104˚C for two hours and reweighed (W2).

$$\text{Solubility (\%)} = \frac{w1-w2}{w1} \times 100$$

2.7 Determination of Biodegradable rate

Soil burial test was performed to analyse the level of deterioration caused to the bioplastics via microbial growth when discharged in to the soil (Wong 2016**).** To determine the biodegradability rate of bioplastic samples, each film sample was pre - weighed (B1) and buried for a month into pots containing conditioned garden soil. After 30 days, the buried sample is taken out from the pot and reweighed. The final weight (B2) of the samples were recorded and the weight loss ratio of the films was calculated by using the following equation:

$$\text{Weight loss (\%)} = \frac{B1-B2}{B1} \times 100$$

B1–Initial weight of film sample
B2 – Final weight of the film after degradation

2.8 Biodegradation test for agar films

Soil burial test was performed to analyze the level of deterioration caused to the bioplastics via microbial growth when discharged in to the soil (Hii et., al 2016) As the base material and plasticizers used in this study, agar, glycerol & sorbitol are known to have hydrophilic properties **(**Vieira et., al 2011) therefore all the samples showed weight loss. However, the type of plasticizer played a pronounced role in the decomposition test. Samples with glycerol plasticizer showed highest weight loss values. when compared to sorbitol. 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 growth of microorganisms which can accelerate the degradation of samples.

3. Results and Discussion

Figure 1: Marine Red Algae - *Gracilaria deblis*

Figure 2: *Gracilaria edulis*

Figure 3: *Gracilaria salicornia*

Figure 4: Gracilaria edulis (Dried)

Figure 5: Gracilaria salicornia (Dried)

Figure 7: Hot water extraction of Agar from *Gracilaria* species

3.1 Extraction of Agar from *Gracilaria* species:

Agar was extracted from the three species of marine red algae – *Gracilaria deblis, G. edulis* and *G. Salicornia* by hot water extraction method.

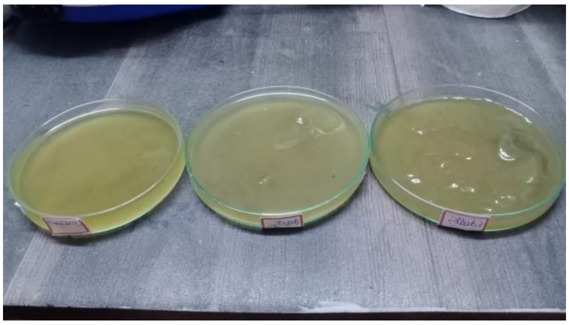

Figure 8: Agar gel formation from *Gracilaria s*pecies after overnight freezing. The agar films obtained from the seaweeds were smooth and clear.

3.2 Determination of agar yield:

Souza et al., 2015 reported that higher yield of agar can be obtained with higher temperature. Other factors affecting the final agar yield are extract time, environment condition, seasonal variations, and physiological factors (PH, Temperature). Some of the popular film forming agents are starch and cellulose derivaties from plant sources other polysaccharides from seaweed (macroalgae) sources such as agar and carageenan Forgetting more attentions due their ability to form edible films as well as the ability at macroalgae to grow in harsh condition.

Yield (%) = $\frac{Dry\ wt.of\ agar}{Dry\ wt.of\ seaweed} \times 100$

Calculation:

1) Dry wt. Of Agar = 18, Dry weight of seaweed = 100
   Yield (%) of *Gracilaria edulis* = $\frac{18}{100} \times 100$ =18%
2) Dry wt. Of Agar = 11.2, Dry weight of seaweed = 100
   Yield (%) of *Gracilaria salicornia* = $\frac{11.2}{100} \times 100 = 11.2$%
3) Dry wt. Of Agar=32, Dry weight of seaweed=100
   Yield (%) of *Gracilaria deblis* = $\frac{32}{100} \times 100 = 32$%

Table 1: Showing agar yield percentage from Gracilaria species

S. No	Name of the Sample	Initial WT (g)	Final WT (g)	Yield of Agar (%)
1	*Gracilaria deblis*	18	100	18%
2	*Gracilaria salicornia*	11.2	100	11.20%
3	*Gracilaria edulis*	32	100	32%

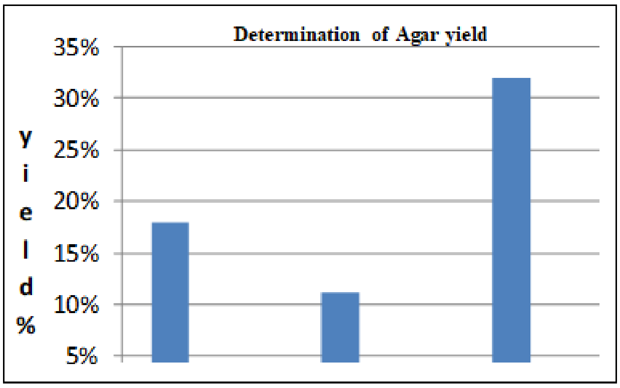

Chart 1: showing agar yield percentage from *Gracilaria* species

3.3 Preparation of Bioplastic of Agar extracted from G. edulis, G. salicornia and G. deblis

Addition of hydrophilic starch, sorbitol, and glycerol in the composition film improves the water absorption capability and hence increases the water activity films and promotes the growth of microorganisms.

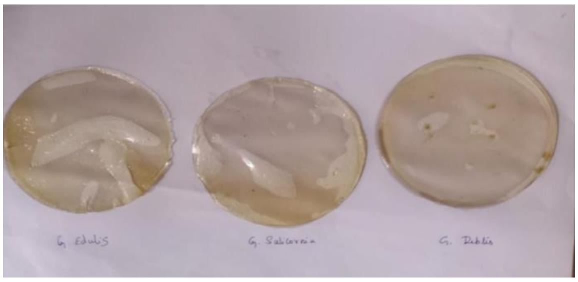

Figure 9: Bioplastic prepared from agar extracted from *Gracilaria edulis, G. Salicornia and G. deblis*

Results showed that the agar film in which glycerol, sorbitol and starch - were added was more flexible and removed easily from the casting plate. Some of the popular film forming agents are starch and cellulose derivaties from plant sources as well as alginate from seaweed sources. Other polysaccharides from seaweed (macro algae) sources such as agar and carageenan are getting more attentions due to their ability to form edible films as well as the ability of macro algae to grow in harsh condition (Siew - ling et al., 2016)

3.4 Determination of Water Solubility Ratio:

In this study three species of *Gracilaria* showed different rate of solubility on testing (Table 2). The present study showed that bioplastic prepared from the agar of *Gracilaria salicornia* was of low strength and has high water solubility when compared with other two species. comparatively *Gracilaria deblis* possess low grade solubility. water solubility reported that 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). Bioplastics having low grade solubility are the best as they resist moisture for a longer period and help to increase the shelf life of products; whereas some edible plastics used in the packaging of food material mostly degrade rapidly (Sanyang et al., 2015). Bioplastics prepared by the addition of sorbitol films were more resistant towards moisture condition.

Calculation:

Weight1 (w1) - Before weight
Weight2 (w2) - After dried

Solubility (%) = $\frac{w1-w2}{w1} \times 100\%$

1. *Gracilaria edulis* (w1) - 0.5, (w2) - 0.08
   = $\frac{0.5-0.08}{0.5} \times 100$ = 84%
2. *Gracilaria salicornia* (w1) - 0.5, (w2) - 0.07
   = $\frac{0.5-0.07}{0.5} \times 100$ =86%
3. *Gracilaria deblis* (w1) - 0.5, (w2) - 0.1
   = $\frac{0.5-0.1}{0.5} \times 100 = 80\%$

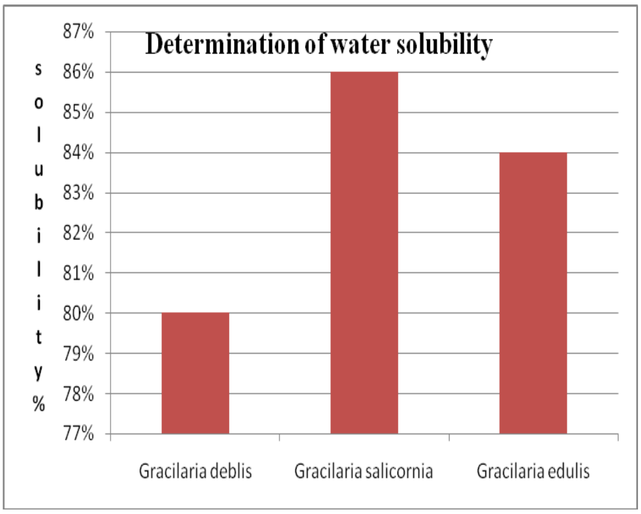

Chart 2: Showing water solubility percentage from *Gracilaria* Species

3.5 Determination of biodegradable rate:

Figure 10: Biodegradation of Bioplastic (Garden Soil)

Figure 11: Weight loss of bioplastics

Figure 12: After 1 month of Degradation (Bioplastics) Calculation;

Weight loss (%) = $\frac{B1-B2}{B1} \times 100$

B1 - Each sample was preweighed

B2 - Final weight of the sample

1. *Gracilaria deblis* B1 - 1, B2 - 0.521

$= \frac{1-0.521}{1} \times 100 = 47.9\%$

2. *Gracilaria salicornia* B1 - 1, B2 - 0.834

$= \frac{1-0.834}{1} \times 100 = 16.6\%$

3. *Gracilaria edulis* B1 - 1, B2 - 0.782

$= \frac{1-0.782}{1} \times 100 = 21.8\%$

The soil burial test was carried to determine the biodegradability of bioplastic prepared from three species of Gracilaria - Gracilaria *deblis, Gracilaria edulis, Gracilaria salicornia.* Results showed that percentange of weight loss of *Gracilaria salicornia* bioplastic was lower when compared with other two species (Table 3). This might due to packaged structure of *Gracilaria salicornia.* The weight loss is higher in the bioplastic prepared from agar extract of Gracilaria *deblis.* The agar having higher sulphated bonds in extended side chains have high hydrophilicity which subsequently promotes microorganism activity (Wong 2016) Addition of hydrophilic starch and glycerol in the composition film improves the water absorption capability and hence increases the water activity films and promotes the growth of microorganisms. The bioplastic prepared from *Gracilaria deblis* exhibited good biodegradability with highest weight loss of 47.9% (Table3) within 30 days of soil burial test

Table 3: Showing biodegradation of percentage of bioplastics prepared from *Gracilaria* species

S. NO	Name of the Sample	Before Weight (W1)	After Weight (W2)	Solubility (%)
1	*Gracilaria deblis*	0.5	0.1	80%
2	*Gracilaria salicornia*	0.5	0.07	86%
3	*Gracilaria edulis*	0.5	0.08	84%

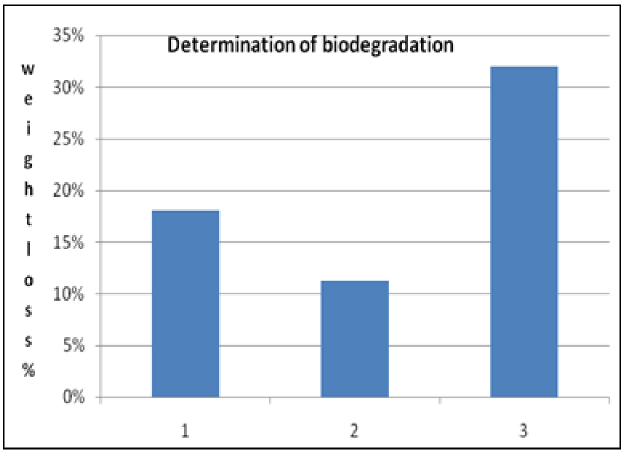

Chart 3: Showing biodegradable rate from *Gracilaria* species

4. Conclusion

Seaweeds films that are formed directly without chemical treatment are a promising approach but currently, the field is still new and more researches are needed. Seaweeds have numerous advantages compared to other biomass, where they do not need pesticide or wide land use while can grow fast, easy to harvest, and cheap. Seaweeds can also be mixed with other seaweed species or materials to improve their characteristics and properties. Hence, using seaweeds as biomass material is a promising approach to replace conventional plastic that can not only contribute to the economy but also eco - friendly.

The agar obtained from red sea weed *Gracilaria deblis* when made into bioplastic using plasticizer, it has shown high rate of biodegradation and low water solubility when compared with other two species but high yield of agar was obtained from *G. edulis*. Bio - plastic films safely breakdown in the environment without leaving any toxicity. As physical and mechanical properties of bio - plastic film meet the properties of conventional plastics, the other properties can be determined in future to use G. edulis to make bioplastic to replace conventional plastics.

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