J. Appl. Biol. Sci., 19 (1): 61-69, January 2025
DOI: https://doi.org/10.71336/jabs.1423
RESEARCH ARTICLE

Journal of Applied Biological Sciences
E-ISSN: 2146-0108; License: CC BY 4.0
Anatolia Academy of Sciences Open Access

Eco-friendly bioplastic from *Pontederia crassipes*: cellulose and carboxymethyl cellulose biopolymers for sustainable, biodegradable bioplastic film

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Received: November 9, 2024
Revised: January 22, 2025
Accepted: January 23, 2025

ABSTRACT: Expanded usage of polyethylene film in different industries lead to accumulation of plastics in the environment, which cause pollution and toxicity to life forms. It can be replaced by the bioplastics which are biodegradable, biocompatible, eco-friendly, and can be obtained from renewable sources at low cost such as plant weeds. The biopolymer, cellulose can be extracted from aquatic weeds since they are abundantly available from aquatic system, easy and safe to handle, and biocompatible. *Pontederia crassipes* an aquatic floating weed, widely dispersed in surface of polluted rivers and ponds. Directed to remove from water bodies by EPA due to its negative impacts on aquatic animal life and the collected biomass as such cannot be used as a feed due to the presence of polyphenols and nitrates. In the current study, about 30% of cellulose was extracted by delignification and bleaching of weed biomass collected from a river. Extracted cellulose was converted into another form of derived polymer, known as carboxy methyl cellulose (CMC) by treating with monochloro acetic acid. Biodegradable bioplastic was prepared using gelatin as gelling agent and glycerol as plasticizer by varying concentrations. Properties of the extracted cellulose, CMC, and bioplastic from cellulose and CMC, were analyzed by measuring film thickness, tensile strength, elongation point, FTIR, and antioxidant assay. About 25.7 µm thickness cellulose biofilm exhibited higher tensile strength of 55.3 MPa at dry, 45.5 MPa at wet condition and 24 µg/ml of antioxidant activity was observed. This research highlights the potential of utilizing water hyacinth, a typically problematic weed, to develop sustainable biopolymers that address the growing environmental concerns associated with conventional plastic waste. Thus, this research work provides a potential biological safe approach to prepare bioplastic film for commercial applications. While, gelatin is costly, a suitable, cheap alternative need to be identified to reduce the cost of production of the bioplastic film. However, further efforts are required to enhance the scalability of the product.

KEYWORDS: Biopolymer, Carboxy methyl cellulose, Cellulose bioplastic, *Pontederia crassipes.*

INTRODUCTION

Plastics play an important beneficial role in food transportation, preservation, hygiene and safety aid, in increasing the lifespan of foods, and nutrition security. The proliferation of single-use or disposable forms in food sector is leading to detrimental environmental impact at local and global scales with potentially negative implications for human health [1]. About 41% of the produced plastic (low-density polyethylene or polyvinyl chloride) is used in packaging, especially in food or beverages. Of the total 6300Mt of plastic waste produced by 2015, only 9% had been recycled or repurposed, with the remaining 91% either incinerated, or placed into landfill or leaking into the natural environment [2].

Petroleum derived plastics used for various human applications because of their desirable characteristics such as lightweight, high durability, and easily available at low cost. Tremendous usage of these plastics led to accumulation of plastics in the environment as municipal wastes which caused environmental toxicity and pollution [3]. Various research works proved that biodegradable plastics are highly capable materials for human applications, by these ways we can reduce the usage of synthetic plastics and prevent the environmental and health risks. By using this concern as platform, diverse biopolymers could be improved into bioplastics like starch, gelatin, cellulose, and chitosan. These polymers can be produced and degraded biologically. To prepare eco-friendly bioplastics, agricultural wastes attracted more and more attention because they are renewable, biodegradable, and biocompatible [4]. Several polymers used for the preparation of polymers, regularly available biopolymer is chitosan, but the purification includes demineralization, deproteinization, decolouration and deacetylation [5], which involves many chemicals and longtime duration for polymer purification. Using starch as

polymer diverted to extract starch from food stuffs these concerns directed to use cellulose as biopolymer. Gelatin was edible protein can be extracted from hydrolysis of collagen which can be dissolved in hot water. Incorporation of gelatin into film preparation would increase the transparency of the biofilms. This could be good material to encapsulate cellulose to form transparent, flexible, and biodegradable films. Various materials were prepared by using gelatin including wound dressing, scaffolds, adhesive and absorbent pads because of their non-toxic and biocompatibility [6].

Cellulose is most abundant, renewable biopolymer exists in plants, wood, cotton [7]. Cellulose can be extracted from bacterial genera includes Gluconacetobacter, Sarcina and Agrobacterium. Compared to bacterial cellulose, cellulose from plants sources considered as significant strategy as these are purified from renewable, readily available sources, and are purified by simple strategy. Cellulose is essential molecule, major component of cell wall. Various other sources were studied including durian rind [4], sugar cane bagasses [8], and corncobs [9] as a source of cellulose for commercial applications. Cellulose can be modified into methyl cellulose, carboxy methyl cellulose, hydroxyl propyl cellulose and hydroxyl ethyl cellulose. With that modification, cellulose has wide range of application in various fields [10]. Carboxymethyl cellulose (CMC) is a cellulose derivative most common form of cellulose. As CMC is water soluble form of cellulose derivative, used in various fields including food industries, pharmaceutical, textiles, and in cosmetic industries as packaging material, stiffening agent, and as thickener. Cellulose in carboxy methylated form would be excellent derivative to support the preparation of biofilm [7].

Pontederia crassipes (water hyacinth) remains as the most widely distributed and vicious aquatic weed. Water hyacinth has become the world's most invasive weed due to its rapid spread, ecological adaptability, and negative impacts causes on the environment, economic development, and human health [10]. It is considered as a menace, as it is very difficult to control its growth in aquatic environment. Removal and disposal of the plant biomass remains as great challenge [11]. Removal of *P. crassipes* from aquatic system helps in reduces the negative impact on aquatic environment, waterways blockage. This approach not only helps in managing waste also create sustainable material that replaces the conventional plastics [12, 13]. Plant biomass consists of 84.5%, 6.5%, 13.03%, 20.6%, and 14.25% of moisture content, ash, crude protein, crude fiber, and dry matter, respectively in water hyacinth. Plant biopolymers (~40%) can be utilized to convert into valuable compostable food packaging films which is useful for the society to replace synthetic plastic material. By utilizing this biomass, the aim is to develop that bioplastics offer effective protection and containment for food products while also being biodegradable and compostable, thus minimizing environmental impact [11].

With growing environmental consciousness and regulations against single-use plastics, businesses and consumers are seeking eco-friendly alternative for extensively used polypropylene plastic material. Thus, the concept of making cellulose based bioplastic from a weed offers a sustainable solution as they break down into natural elements, reducing pollution and landfill waste. This trend aligns with India push for greener practices in packaging, fostering a market for innovative, biodegradable packaging options. The global shrink film market size valued as $4.7 billion in 2021, and expected to grow at a compound annual growth rate of 5.4% from 2022 to 2031 [14]. Current study planned with an objective of preparation of bioplastic film from extracted cellulose from *P. crassipes*. Ultimately, the goal is to provide a viable alternative to traditional plastics, reducing pollution and contributing to a more sustainable packaging solution.

MATERIALS AND METHODS

Collection of plant biomass as substrate

Pontederia crassipes plant were collected and stored in a sterile polyethylene bags from Noyyal River in Tirupur, Tamilnadu, India (latitude of 11°11'63.77"N Longitude of 77° 30' 58. 99" E). Collected plant was identified, verified and authenticated by Scientist 'F' & Botanist Dr. M. U. Sharief, Botanical survey of India, Tamilnadu Agriculture University based on plant size, leaf size, structure and color, flower color and arrangement, stem structure and root system in the certificate number of BSI/SRC/5/23/2024-25/Tech-339 as *Pontederia crassipes*. Plant authentication provided comprehensive and reliable confirmation of the plant species used in this research. Collected plant biomass was thoroughly washed with distilled water twice and shade dried. The shade dried biomass was ground into fine powder by sterile blender and stored in an airtight container for further use.

Extraction of cellulose

To extract cellulose from plant biomass, delignification and bleaching were performed. For delignification, about 5g of leaf powder mixed with 100 ml of 5M sodium hydroxide solution, mixed in a magnetic stirrer at 60˚C for 2h. Obtained hydrolysate was filtered using Whatmann no. 1 filter paper to remove chlorophyll and plant residues, the above process repeated until to get clear filtrate. Then for bleaching, 5% of sodium hypochlorite mixed with the filtrate, stirred for 20min and the same process repeated to remove lignin and hemicellulose completely. After final bleaching, obtained

biomass was filtered, washed with distilled water, and neutralized to reach pH 7. Extracted cellulose was dried at 45˚C for 24h and stored for further analysis [15].

Characterization of cellulose

To determine the microstructure of cellulose, purified cellulose powder observed under optical microscope at 10X, 40X, and 100X magnification. To analyses the water retention capacity of cellulose (WRC), about 2g of cellulose mixed with 30ml of distilled water and allowed to absorb water for 10 minutes. After 10 minutes, solid biomass centrifuged at 2000 rpm for 15 minutes and pellet collected [16]. Wet weight of sample measured and WRC calculated using formula;

$$\text{Water retention capacity (WRC)} = \frac{\text{Weight of the sample after absorption of water (g)}}{\text{Weight of dry sample}} \times 100$$

Eqn: 1 Water retention capacity calculation formula

Preparation of carboxymethyl cellulose (CMC) from cellulose

To convert cellulose into carboxymethyl cellulose, 5g of cellulose mixed with 25 ml of 15% sodium hydroxide and allowed to stand for an hour. To the mixture, concentrated monochloroacetic acid added drop wise, stirred at 55˚C for 3h, followed by filtration and neutralized. The obtained slurry dried at 60˚C in an oven for 6 hours [17].

Preparation of biofilm from cellulose

Cellulose films prepared by casting method at room temperature. Exactly 0.2g of gelatin mixed with 10ml of distilled water and dissolved at 80˚C for 15 min. To the slurry 0.1ml of glycerol was added and solution casted on a glass plate and allowed to dry at room temperature for 24 hours. After complete drying, films were peeled off from glass plate and stored for further use. Instead of 2% cellulose, replaced with 2% CMC to prepare CMC based bioplastic. For standardization of gelatin concentration, 0.5%, 1%, 1.5%, 2%, 2.5%, and 3% of gelatin was added to 10ml of distilled water with 2% cellulose to determine the effect of gelatin on biofilm formation. Similarly, instead of 1% of glycerol, 0.5%, 1%, 1.5%, 2% of glycerol was added to the solution to evaluate the ability to form biofilm [18].

Analysis of biofilm properties

Thickness of the biofilm measured using digital micrometer. About 3 different measurements were taken from different positions of the film. From 3 measurements, average value calculated and expressed in micrometer [19]. Tensile strength of the biofilm was analyzed by Zwickroell Universal strengthtester-5KN [20].

Fourier transform infrared analysis

Functional groups and molecular bonds of extracted cellulose, CMC, and for prepared biofilm (with cellulose and CMC) identified by JASCO FT/IR-460 Fourier transform infrared spectrophotometer. Spectra were recorded range at 4000-400 cm^{-1} with a resolution of 4 cm^{-1} [21].

Determination of antioxidant activity

About 6mg of DPPH were mixed with 50ml of ethanol to prepare DPPH solution. To prepare sample, 50 mg of cellulose/CMC/biofilm mixed with 5ml ethanol. To 2.5 ml of each sample, mixed with 2.5 ml DPPH solution, and incubated for 30 minutes under dark. Standard curve was plotted using ascorbic acid as positive control and absorbance was measured at 517 nm [22].

RESULTS AND DISCUSSION

Collection of plant biomass as substrate

Plant biomass was collected from Noyyal River, Tiruppur was shown in Figure 1. Identified and authenticated as *P. crassipes* with the certificate number of BSI/SRC/5/23/2024-25/Tech-339. From 25g of dried *P. crassipes* biomass 24.38 g of fine powder was obtained (Figure 2). Cellulose content in *P. crassipes* measured as high (16–64.51 (wt. %)) compared to other weeds such as *Amaranthus* sp., (44%), *Chenopodium album* L. (23% to 53%), *Aloe vera* (~ 60%), *Coccinia grandis* (63%), and *Parthenium hysterophorus* (27% to 36.43%) [10]. Thereby, *P. crassipes* plant biomass can be exploited for the extraction of cellulose.

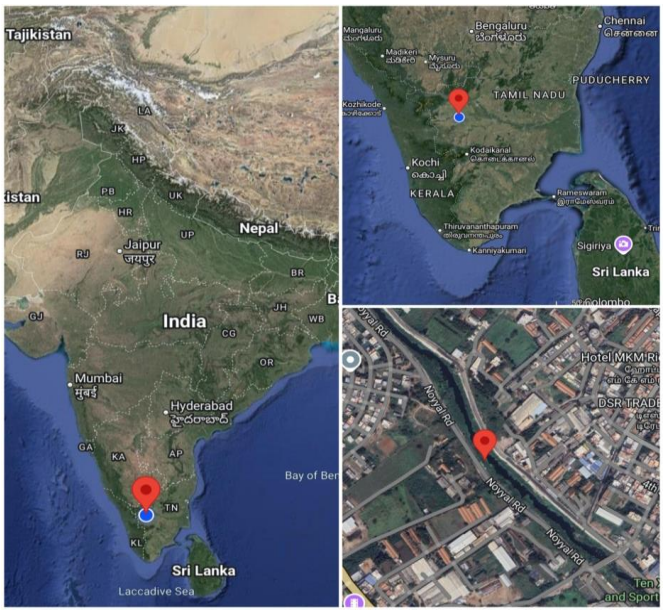

Figure 1. *Pontederia crassipes* plant biomass collected site

Extraction of cellulose

Cellulose extracted after 24 hours of drying. White colour, semi-transparent, crystalline powder obtained. Alkaline hydrolysis with sodium hydroxide lyses lignin in plant biomass. Sodium hypochlorite breaks the long hemicelluloses chains which were further removed by bleaching [23]. Plant chlorophyll and other precipitated plant residues removed by filtration, thereby a white to cream colour residue obtained. Figure 2 showed process of cellulose extraction. White color indicated the removal of hemicellulose and lignin which was previously acknowledged by Packiam et al. [21] and Umesh et al. [24] that treatment of plant biomass with sodium hypochlorite result in high yield of cellulose compared to other chemicals. About 0.3g of cellulose was obtained from a gram of leaf dry biomass. Yield of cellulose from the sample calculated using the following formula;

Cellulose yield = Dried cellulose mass ÷Initial mass of leaf powder × 100

$$\text{Cellulose yield} = \frac{0.3}{1} \times 100$$

This means, cellulose yield was about 30% [18] also similar study in *P. crassipes* and concluded that cellulose yield as 28.5%.

Cellulose content is high in root part of the plant. As whole biomass used for the cellulose extraction resulted in 30% of yield. To improve cellulose yield, the study recommends using the whole plant biomass for maximum output. Consequently, removing the weed enhances the survivability of fish, increases the decomposition of organic materials, improves fishing conditions in water bodies, prevents water loss through evapotranspiration by the weed, and allows for the effective use of water for irrigation purposes [25].

Characterization of cellulose

Extracted cellulose found as small fibers in ~ 50 µm length and ~ 30 µm breadth (Figure 3). Benitez et al [20] also reported cellulose from *P. crassipes* was relatively larger in size while compared to chemical cellulose. Water retention capacity (WRC) analysis of extracted cellulose showed that about 1g of cellulose able to absorb 2.3g of water whereas, chemical cellulose able to absorb 1.9 g water. Compared to chemical cellulose, extracted cellulose absorbed more water and swell. Umesh et al. [24] interpreted that WRC is preferable property in formation of texture in packaging materials.

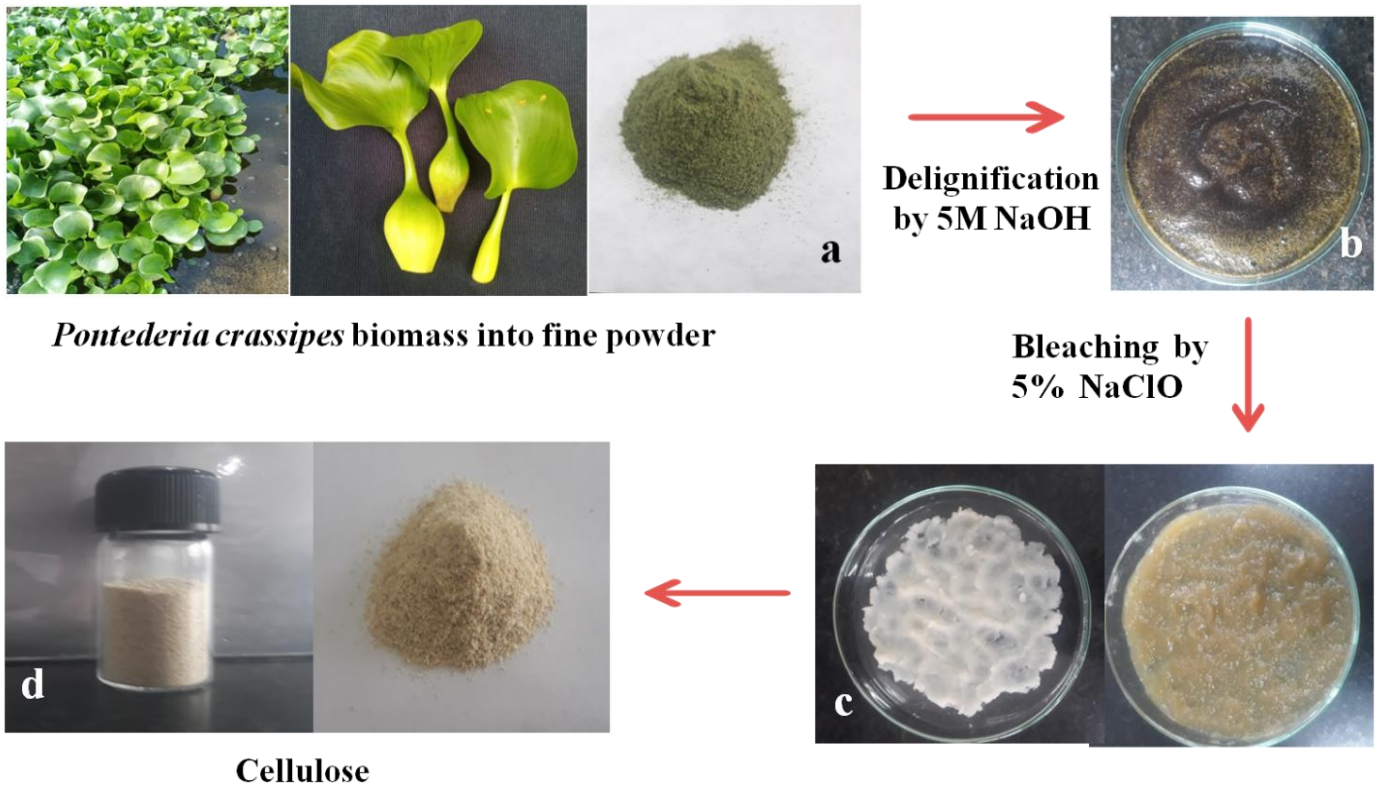

Figure 2. Cellulose extraction process. a) Preparation of dry plant biomass, b) Delignified plant biomass, c) Bleached biomass before drying, d) Dried, pure cellulose from plant biomass.

Preparation of CMC from cellulose

After drying 3.5 g of white colored, crystalline CMC powdered was obtained and stored for further use. Preparation of CMC was done by alkalization and carboxymethylation of cellulose. Treating cellulose with NaOH produce alkali cellulose and carboxymethylation by monochloro acetic acid produce CMC by substitution process with glycolic acid as byproduct. Neutralizing CMC with 100% methanol or ethanol eliminates glycolic acid. Putri et al [26] prepared CMC through alkalization and carboxymethylation and reported as NaOH and monochloroacetic acid induced the CMC preparation.

Preparation of bioplastic film from cellulose

Cellulose biofilm was successfully prepared by casting method which was clear, transparent, smooth and flexible shown in figure. 4. Cellulose was a polysaccharide formed by β (1-4)-D-glycosidic units due to its polymeric nature dissolution of cellulose would be a difficult process. Instead of dissolving, cellulose was encapsulated in gelatin polymer matrix which supports for mechanical properties of the film. For CMC, dissolved in water and bioplastic was prepared. Glycerol was responsible for the plasticizing ability of the film. Navasingh et al. [4] reported the possibility to prepare transparent and flexible biofilm from biological cellulose. Benitez et al. [20] concluded that cellulose encapsulation by gelatin matrix, form a transparent and flexible bioplastic film.

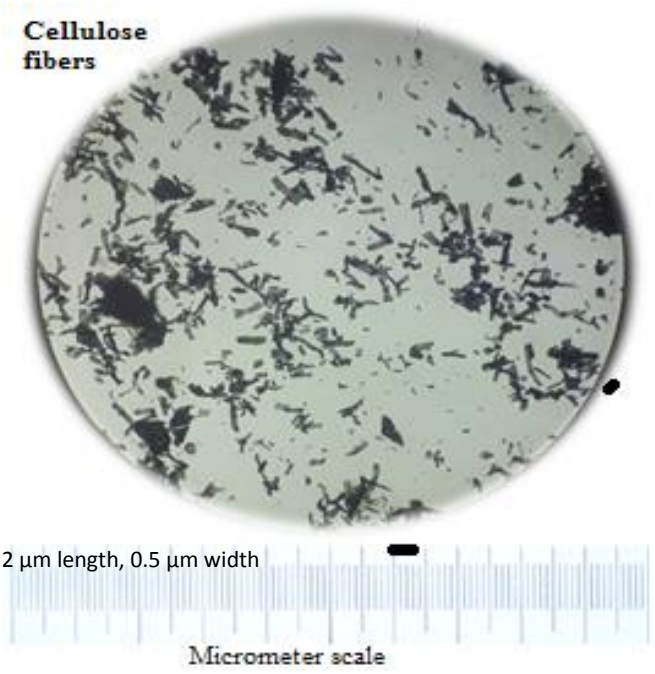

Figure 3. Extracted cellulose fibers under 100X objective and Stage micrometer (each 1 division is 100µm)

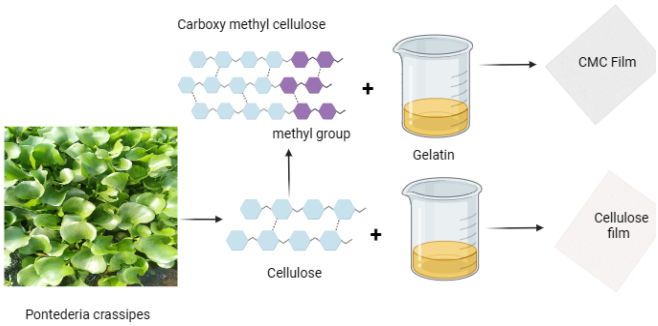

Figure 4. Diagrammatic representation of biofilm preparation from *P. crassipes*

Effect of gelatin on bioplastic formation

Compared to other concentration 2.5% of gelatin able to form thin film (Figure 5). Gelatin concentrations of 0.5, 1, 1.5 and 2% resulted in the preparation of too thin and highly flexible plastic film with low stability. Whereas 2.5% gelatin able to form thin, solid, and flexible film. When gelatin concentration increased, yielded durable film. At 3% and above concentration formed a solid film. Exactly at 2.5% concentration able to form flexible, smooth, and solid bioplastic film. Gelatin is an excellent gelling agent which melts at 28-31˚C and form gels at 20-25˚C and the films have been extensively studied because of its low cost, film uniformity, and outstanding optical properties [27]. Pan et al [18] prepared composite films with gelatin and reported its potent antimicrobial activity.

Effect of glycerol on biofilm

Compared to other concentrations, 1% of glycerol able to form flexible film (Figure. 6). Low concentration of glycerol resulted in the formation of opaque and highly brittle bioplastic film, high concentration of glycerol resulted in highly sticky bioplastic film. So that 1% of glycerol considered as the optimum for the formation of transparent, flexible, and clear film [18] with glycerol and also recorded that 1% glycerol resulted in flexible and smooth bioplastic film.

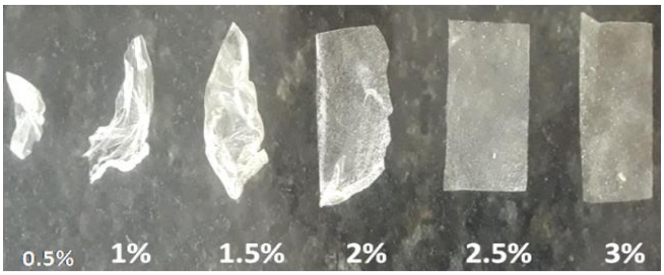

Figure 5. Bioplastic preparation from cellulose biomass in combination with 0.5%, 1%, 1.5%, 2%, 2.5%, and 3% of gelatin

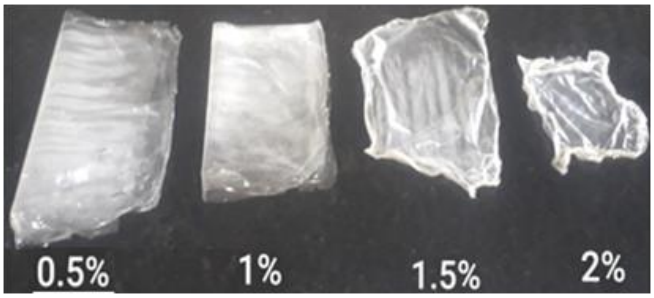

Figure 6. Bioplastic preparation from cellulose biomass in combination with 0.5%, 1%, 1.5%, and 2% of glycerol

Analysis of biofilm properties

By calculating the averages, thickness of the CMC film was 26.6 µm and cellulose film was 25.7 µm. Navasingh et al [4] reported that thickness of their cellulose film was 23 µm which was correlated with our results. Onyeaka et al. [6] acknowledged nanocellulose-gelatin film thickness as about 28 µm in thickness.

Tensile strength

Tensile strengths of prepared biofilm were shown in table 1. The average tensile strength and elongation point of CMC film and cellulose film were 43.2, 55.3 MPa and 1.7% and 1.9% at dried state respectively. Strength of cellulose bioplastic film was 10% higher than CMC film and elongation point also slightly higher than CMC film. Strength of cellulose film in swollen form was slightly lower but elasticity was significantly increased due to hydrogen bonding between gelatin and water molecules, which increased the elasticity of the bioplastic film. Compared to CMC film, cellulose film showed higher tensile strength and elasticity. Interaction of cellulose with gelatin gave more stability than CMC. Nguyen et al. [28] also observed similar result in their study that bioplastic films from cellulose polymer exhibited higher tensile strength and elasticity.

Table 1. Tensile strengths of bioplastic films

Film type	Tensile strength in MPa		Elongation point (%)	
	Dry	Wet	Dry	Wet
CMC bioplastic film	43.2	40.0	1.7	15.4
Cellulose bioplastic film	55.3	45.5	1.9	20.5

Fourier transform infrared spectrophotometric analysis

FTIR analysis showed the presence of seven different functional groups in the extracted cellulose and CMC (Figures. 7, 8; table 2) between 4000 and 400 cm^{-1}. Peaks at 3747.98 cm^{-1} and 3336.25 cm^{-1} indicated O-H stretch, free hydroxyl due to the presence of alcohols and phenols. A peak at 2920.66 cm^{-1} would indicate C-H Stretch of alkanes. A strong peak at 1615.09 cm^{-1} denoted C=C stretch of alkenes. A peak at 1417.42 cm^{-1} indicated C-C Stretch in ring of Aromatics. Medium peaks at 1217.14 cm^{-1} and 1012.46 cm^{-1} indicated C-O Stretch of alcohols, carboxylic acids, esters and ethers. Based on the FTIR analysis, bonds of C-C, C-O, and C-H would represent the characteristics of cellulose. Salim et al. [29] reported that molecular bonds of C-H and O-H were main functional groups of cellulose. Observed peaks were correlated with chemically synthesized control cellulose functional groups. Peak at 1109 cm^{-1} and 1121 cm^{-1} indicated the addition of carboxyl group from this able to confirm formation of CMC from cellulose. Other peaks represented functional groups of cellulose.

FTIR spectra and corresponding functional groups of cellulose film and CMC film was shown in Figures 9, 10 table 3. Peaks of cellulose films at 1639 cm^{-1}, 1628 cm^{-1} and 1540 cm^{-1} due to the presence of gelatin indicated by the C=O stretch vibration. All the peaks would represent the interactions between of cellulose and gelatin which point out a good molecular compatibility between cellulose and gelatin film. Same functional groups were reported by Onyeaka et al [6].

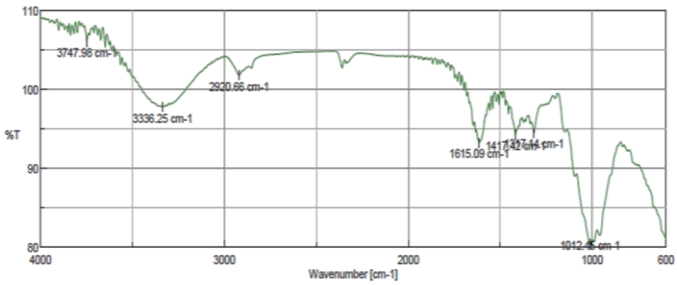

Figure 7. FTIR spectra of cellulose from *P. crassipes*

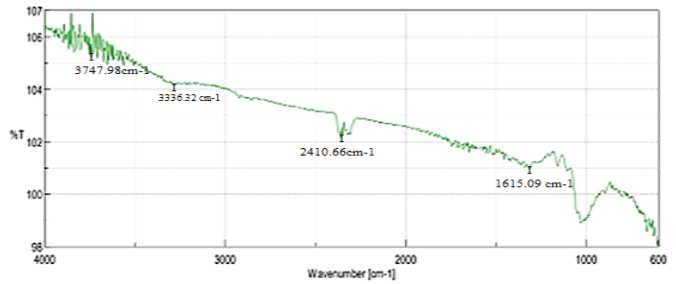

Figure 8. FTIR spectra of carboxyl methyl cellulose

Table 2. Functional groups in extracted cellulose and CMC

Obtained peak frequency in cm^{-1}		Reference groups	Functional groups
Extracted cellulose	CMC		
-	3896	O-H Stretch, Free hydroxyl	Alcohols, Phenols
3747.98	3747.98	O-H Stretch, Free hydroxyl	Alcohols, Phenols
-	3740	O-H Stretch, H- bonded	Alcohols, Phenols
-	3620	O-H Stretch, Free hydroxyl	Alcohols, Phenols
3336.25	3336.25	O-H Stretch, H- bonded	Alcohols, Phenols
2920.66	-	C-H Stretch	Alkanes
1615.09	-	C=C stretch	Alkene
1417.42	-	C-C Stretch (in ring)	Aromatics
1217.14	-	C-O stretch	Alcohol, Carboxylic acids, Esters, Ethers
-	1121	C-H Wag	Alkyl halides
-	1109	C-H Wag	Alkyl halides
1012.46	-	C-O Stretch	Alcohol, Carboxylic acids, Esters, Ethers
-	810	=C-H Bend	Alkenes

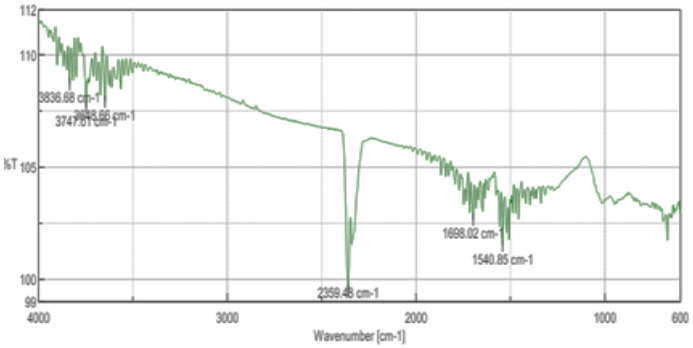

Figure 9. FTIR Spectra of cellulose film

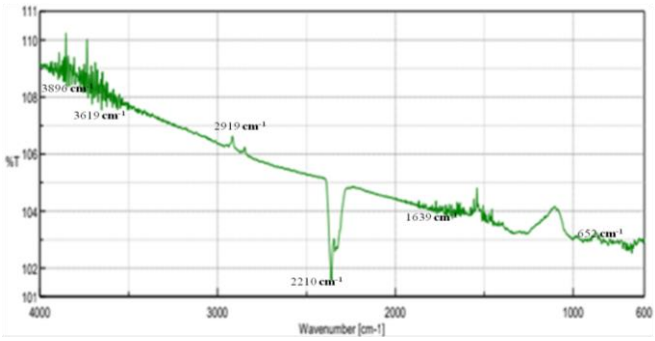

Figure 10. FTIR Spectra of CMC film

Table 3. Functional groups of cellulose film and CMC film

Obtained peak frequency in cm^{-1}		Reference groups	Functional groups
Cellulose film	CMC film		
-	3896	O-H Stretch, Free hydroxyl	Alcohols, Phenols
3836.38	-	O-H Stretch, Free hydroxyl	Alcohols, Phenols
3747.01	-	O-H Stretch, Free hydroxyl	Alcohols, Phenols
3648.66	-	O-H Stretch, Free hydroxyl	Alcohols, Phenols
-	3619	O-H Stretch, Free hydroxyl	Alcohols, Phenols
-	2919	C-H Stretch	Alkanes
2359.48		O=C=O	Carbon dioxide
-	2210	-C= C- Stretch	Alkynes
1698.02	-	C=O Stretch	Carboxylic acids
-	1639	C=O Stretch	Carboxylic acids
1540.85	-	C-C Stretch (in ring)	Aromatics
-	652	-C-H bend	Alkynes

Determination of antioxidant activity

To detect the presence of antioxidant property of the prepared polymer and its bioplastic film, DPPH assay performed. Results of antioxidant activity were showed in table 4. When DPPH reacts with antioxidant compounds accept electron and changes its color from violet to yellow [20]. Cellulose showed very low level of antioxidant activity whereas gelatin showed 5% of increased activity compared to cellulose. But cellulose and CMC reacts with gelatin it exhibits low antioxidant activity. Reduction in oxidation process extends the shelf life of food. Pirsa et al. [30] reported similar findings for antioxidant activity of cellulose and CMC films.

Table 4. Results of antioxidant activity

Name of the component	Concentration of antioxidants in µg/ml
Cellulose	24
Gelatin	106
CMC	20.2
Cellulose-gelatin film	50.2
CMC-gelatin film	43.7

CONCLUSION

From the above described research work, concluded that 30% of cellulose can be extracted from the biomass of *P.crassipes* by the process of alkaline hydrolysis. Extracted cellulose visually appeared as half white colour, crystalline structure. Conversion of cellulose to CMC carried out using monochloro acetic acid. Cellulose and CMC were converted to bioplastic film successfully by casting method into a smooth and flexible plastic film. A thin, transparent, and flexible bioplastic films were obtained. From FTIR analysis of biofilm, indicated the presence of seven different functional group with highly reactive OH stretch. Overall, this study was a proof that prepared cellulose biofilm exhibited high tensile strengths, elongation property, and antioxidant activity. This innovative approach in preparation and usage of bioplastic could reduce the spread of synthetic plastics and align with the demand for natural products. These kinds of biocompatible, non-toxic, and nutritional value having bioplastic can be a trend setter in food industry by replacing synthetic plastic and enhance the shelf life of perishable foods. Investigating the antimicrobial activity of cellulosic films against various foodborne pathogens highlights their potential role in combating such pathogens. These films can serve as food packaging materials, leveraging their antimicrobial properties effectively.

DECLARATION

Acknowledgement

The authors express their gratitude to Rathnavel Subramaniam (RVS) College of Arts and Science for providing laboratory facilities to conduct our research work. Special thanks to the DST-FIST, Government of India, for providing established instrumentation facilities to complete the work at RVS College of Arts and Science, Coimbatore, India.

Authorship contributions

Conceptualization, Data curation, and Supervision: P. Saravana kumari; Biological analysis and chemical analysis: Abiyoga M.

Funding

The present research received no grant from any funding agency.

Consent for publication

All authors read and approved the final manuscript.

Competing interests

The authors declared that there is no conflict of interest.

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