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Development of Sustainable Cellulose-Based Biopolymer Sheets from Water Hyacinth (*Pontederia crassipes* (Mart.) Solms): Extraction, Characterization, Fabrication, and Performance Evaluation

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Abstract

Objectives: This study aimed to extract cellulose from water hyacinth (*Pontederia crassipes* (Mart.) Solms) Extract cellulose using three different methods, describe the extracted cellulose, and produce cellulose-based biopolymer sheets for sustainable use. **Method:** Three chemical treatment methods were used for the extraction of cellulose, Alkaline treatment, Bleaching. The extraction yields were compared, and the selected cellulose was characterized using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). The sheets made of cellulose were produced using distilled water and 1% starch and functional properties were compared to commercial cellulose sheets. **Findings:** The extraction method I yielded the maximum cellulose content (35.5 $\pm$ 0.24%) while the extraction method II and III yielded 24 $\pm$ 0.12% and 8 $\pm$ 0.33% respectively. The crystallinity index obtained from XRD was 87.79% and the diffraction peak was prominent at 22.455°. A significant degradation event was found between 350.5 and 505.4°C, with a weight loss of 60.53%, by TGA. The fabricated sheets showed better water retention capacity and oil retention capacity under the test condition than commercial cellulose. **Novelty**: This research not only compares the performance of the three methods of extracting the cellulose from water hyacinth but also combines the extraction, physicochemical characterization, sheet fabrication, and evaluation of functional properties. The results prove that invasive aquatic plants can be a desirable renewable cellulose source for producing sustainable biomaterials. Additional studies are needed to determine the mechanical strength, biodegradability and barrier properties of this product before it is applicable in practice.

Keywords: Water hyacinth (*Pontederia crassipes*); Cellulose-rich fibre; Biopolymer sheets; X-ray Diffraction (XRD); Thermogravimetric Analysis (TGA)

1 Introduction

The valorisation of renewable biomass into biodegradable materials is becoming more and more relevant for sustainable material development and circular bioeconomy programs. In terms of potential resources, there are aquatic weeds that can cause ecological disturbances such as water blocking, decrease water quality and impact aquatic biodiversity, namely water hyacinth (*Pontederia crassipes*). Yet, its high level of biomass that can be cultivated in a fast and sustainable manner and is composed of cellulose, presents a chance to utilize an environmental challenge as a beneficial raw material for sustainable material production [1], [2], [3], [4].

The water hyacinth has been investigated for cellulose extraction and cellulose nanofibres, nanocrystals, biodegradable films and composites preparation in the past [5], [6]. The investigations show its potential as an alternative source of lignocellulose. However, additional studies are required to enable the extraction of cellulose and the systematic physicochemical characterization and fabrication of biopolymer sheets in a single workflow. Furthermore, the effect of extraction and bleaching treatments on the yield of cellulose and its structural properties and thermal stability is crucial for the evaluation of its applicability for practical use [7], [8].

The present study is aimed to tackle these aspects by studying the cellulose-rich fibres isolation from P. crassipes using alkali and bleaching treatments followed by its characterization using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffraction (XRD) and thermogravimetric analysis (TGA). The cellulose extracted was then used to form biopolymer sheets. The integrated approach combining extraction optimization, comprehensive characterization and sheet fabrication would enable the utilization of the aquatic biomass of invasive species for the production of value-added biodegradable materials, thus promoting sustainable resource management and the principles of circular bioeconomy.

2 Methodology

2.1 Substrate collection

The collection of Water hyacinth was done from Madambakkam lake, Madambakkam, Chennai, Tamil Nadu. Plants of water hyacinth were removed and cleaned and stored for drying, except for their roots. At this stage it has a water content of 94.6 %. The fresh water hyacinth plants were dried for 3 days in hot air oven at 60°C to eliminate extra water in the plants. The dried water hyacinth was powdered and stored for extraction and further analysis (Figure 1 a & b).



Fig 1. a) Water hyacinth after sun and oven dried ; b) Water hyacinth powder

2.2 Extraction of cellulose fibers from water hyacinth

2.2.1. Method – I

For optimum concentrations of alkalis, 150 ml of 10% sodium hydroxide solution was applied to the water hyacinth powder for 120 minutes at 70°C. For each extraction replicate, 10 g of dried water hyacinth powder was used. The treatment was repeated for three cycles using freshly prepared 150 mL of 10% sodium hydroxide solution for each cycle. The treated material was then filtered, washed with tap water and finally with distilled water till neutral pH was obtained. Bleaching was then undertaken with 200 mL of bleaching solution containing 2% sodium chlorite and 2% glacial acetic acid at 80°C for 120mins twice. The sample was washed with distilled water after each bleaching cycle to neutralize the pH. Lastly, the bleached fibers were dried using a hot air oven at 60°C until constant weight was achieved [8].

2.2.2. Method – II

For each extraction replicate, 10 g of dried water hyacinth powder was treated with 150 mL of 4% sodium hydroxide solution for 240 minutes at 80ºC. The treatment was repeated for three cycles using freshly prepared 150 mL of 4% sodium hydroxide solution for each cycle. The treated material was then filtered, washed with tap water and then with distilled water until the pH of the material was neutral. Bleaching then was done with 200 mL of 4% sodium hypochlorite solution at 30°C for 180 minutes, and then again. The sample was washed with distilled water after each cycle of bleaching, to neutralize the pH. Lastly, the bleached fibers were hot air dried at 60°C[8].

2.2.3. Method – III

For each extraction replicate, 10 g of dried water hyacinth powder was used. The water hyacinth powder was subjected to three treatment cycles using freshly prepared 150 mL of 4% sodium hydroxide solution for each cycle at 60°C for 120 minutes in order to increase the alkali concentration. The residue was washed with distilled water till it was neutral. A further bleaching procedure was then undertaken twice with 200 mL of 4% Hydrogen peroxide (H2O2) in 4% sodium hydroxide (NaOH) at 80ºC for a total of 120 minutes. To neutralize the pH of the bleached sample, distilled water was used for washing and then the sample was dried in a hot air oven at 60°C[8]. All three extraction methods were performed in triplicate (n = 3).

Yield calculation:

$$Cellulose\ yield\ (\%) = \left(\frac{Weight\ of\ dried\ cellulose-rich\ fibres\ recovered\ (g)}{Initial\ weight\ of\ dried\ water\ hyacinth\ powder}\right) \times 100$$

2.3 Analysis of functional properties of the extracted cellulose

2.3.1. Bulk Density

The volume of the dried cellulose measured in a 10 mL measuring cylinder after gentle shaking was used to determine the bulk density following TAPPI standard procedure[9].

$$Bulk\ density\left(\frac{g}{mL}\right) = \left(\frac{amount\ of\ sample(g)}{volume\ of\ sample(mL)}\right)$$

2.3.2. Packed Density

The packed density was calculated by placing 0.24 g of the dried cellulose in a 5mL graduated syringe and then compacting it using the piston until no increase in pressure is felt[9].

$$Packed\ density\left(\frac{g}{ml}\right) = \left(\frac{amount\ of\ sample\ (g)}{least\ volume\ of\ sample\ (mL)}\right)$$

2.3.3. Hydrated Density

The hydrated density was measured by placing 0.241g of dry cellulose into 5mL of distilled water in a 10mL measuring cylinder. The amount of water displaced by cellulose was determined[9].

$$Hydrated\ density(g/mL) = \left(\frac{sample(g)}{mL\ of\ water\ displaced}\right)$$

2.3.4. Water Retention Capacity (WRC)

To determine water retention capacity (WRC), 2g of extracted cellulose was mixed with 30 mL of distilled water in a 50 mL centrifuge tube. The slurry was allowed to settle for 10 minutes and then centrifuged at 2000 rpm for 15 minutes. After draining off the supernatant, the wet weight of the cellulose pellet was then recorded[9].

$$Water\ Retention\ Capacity\ (g/g) = \left(\frac{weight\ of\ water\ (g))}{weight\ of\ dry\ sample\ (g)}\right)$$

2.3.5. Oil Retention Capacity (ORC)

The method for oil retention capacity (ORC) was similar to that of water retention capacity (WRC) with palm oil used instead of water[9].

$$Oil\ Retention\ Capacity\ (g/g) = \left(\frac{weight\ of\ oil(g))}{weight\ of\ dry\ sample(g)}\right)$$

2.3.6. Emulsifying Activity (EA)

7 g of the cellulose extracted was dissolved in 100 mL of water and then in 100 mL of soybean oil. The mixture was then homogenized at 1000 rpm for 1 minute. The emulsion went through a centrifuge at 1300 rpm for 5 minutes[9].

$$Emulsifying\ Activity = \left(\frac{Height\ of\ emulsifier\ level\ (cm)}{Height\ of\ whole\ layer\ (cm)}\right) * 100$$

2.4 Characterization of extracted cellulose

Various techniques were employed to determine the functional and structural properties of water hyacinth based cellulose. Functional groups (4000-400 cm-1) were analyzed using FTIR spectroscopy (Shimadzu IR Spirit-L)[10]. The morphology was studied by SEM (Jeol JSM 6390)[11]. The purity and crystal phase were determined by XRD (Shimadzu XRD 6000)[12]. DSC (Netzsch DSC 204 F1) analyzed thermal properties (-50°C to 400°C at 10°C/min)[7]. Thermal stability (25°C to 800°C) was determined by TGA (Perkin Elmer STA 6000)[7]. The analyses gave useful information about the physicochemical properties of the cellulose extracted, helpful in assessing their applications[13].

2.5 Fabrication of cellulose sheets

The cellulose sheet was first created by mixing 2.5 g of extracted cellulose with 100 mL of distilled water and stirring the mixture for 6 hours. The solution was then homogenized at 7400 rpm for 20 minutes and allowed to stand overnight in a cold room at 4 °C. The next step was to re-homogenize the sample at the same conditions with the addition of a plasticizer (1% starch). This mixture was then transferred into an aluminium tray and incubated at 60 °C[13].

2.6 Statistical Analysis

All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation (SD). The mean and standard deviation were calculated from the three independent experimental readings for each extraction method. The obtained values were used to assess the consistency and variability of the experimental results. Statistical analysis was performed using descriptive statistics.

3 Results and Discussion

3.1 Extraction of cellulose

The three extraction methods for this study were evaluated for cellulose isolation based on cellulose recovery [Figure 2a & b]. The cellulose yields were 35.5 ± 0.24%, 24 ± 0.12%, and 8 ± 0.33% for Methods I, II and III, respectively [Table 1]. Method I gave the maximum yield and hence was chosen for further characterization and fabrication of biopolymer sheets.

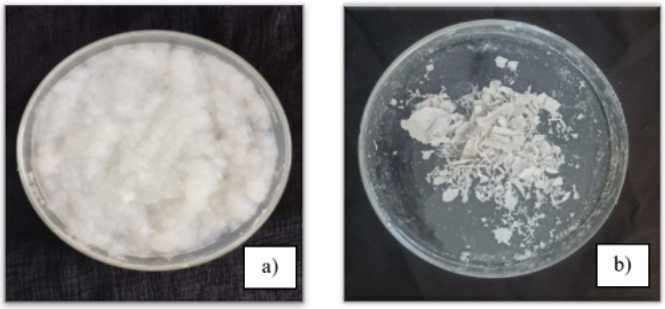

Fig 2. a) Extracted cellulose fibre before drying; b) Extracted cellulose fibre after drying

The higher recovery obtained by Method I indicates that sequential alkali and bleaching treatment is efficient to remove lignin and hemicellulose, leaving the cellulose fraction. The suitability of water hyacinth as a raw material for cellulose production by chemical and mechanical pre-treatment has been demonstrated in previous studies, and this technique can also be used to produce nanocellulose[14]. The studies reported above suggest that water hyacinth can be used as a source of cellulose, while this work is directed towards high-yield production of cellulose and immediate utilization in the form of a biopolymer sheet.

Table 1. Percentage of cellulose from water hyacinth using three different methods

Method	Bleaching agent used	% Yield of Cellulose (w/w)
Method 1	2% Sodium chlorite and 2% Glacial acetic acid	35.5 ± 0.24 %
Method 2	4% Sodium hypochlorite	24 ± 0.12%
Method 3	4% hydrogen peroxide and 4% sodium hydroxide	8 ± 0.33%

The difference among the three methods further illustrates the need for the optimization of the extraction process. Method I gave better recovery of 35.5 ± 0.24% as compared to Method II and Method III where 24 ± 0.12% and 8 ± 0.33% were obtained respectively. So, Method I was the most efficient method of the methods tested. The optimized cellulose was then characterized in terms of functionality, structure and thermal properties prior to the sheet fabrication.

Water Hyacinth has been studied for its cellulose and nanofibrillated cellulose containing potential with various methods of extraction and processing techniques [15], [16]. The studies show the potential of water hyacinth in producing materials based on cellulose by chemical and mechanical processing. The three extraction methods compared in the present study yielded the highest cellulose content of 35.5 ± 0.24%, Method I, followed by Method II (24 ± 0.12%) and Method III (8 ± 0.33%). The yield with Method I was 48.0% more than Method II and 344% more than Method III. These results suggest that Method I is the best procedure of the three methods studied. In the present work, comparison of extraction performance with functional evaluation was correlated with physicochemical characterization and further transformation in the form of biopolymer-sheet fabrication, unlike the studies focusing on cellulose or nanofibrillated cellulose production [15], [16]. However, direct comparisons between superiority of this method and that previously reported needs similar yield and processing data.

3.2 Functional properties of extracted cellulose

The extracted cellulose was compared with commercial cellulose (CC) in terms of functional properties [Table 2]. The bulk, packed and hydrated densities of the extracted cellulose were higher than those of commercial cellulose. Moreover, water retention capacity (WRC) and oil retention capacity (ORC) were also found to be higher than commercial cellulose and some emulsifying activity was also discerned.

The difference in the values show good surface and hydration properties of the cellulose which has been extracted. Hydrophilic hydroxyl groups and the porous fibrous structure formed during chemical treatment may be related to the higher WRC. Likewise, the increased ORC could be due to surface area and physical properties of the fibres. It is important that the biomaterials have such characteristics to be cellulose-based since they affect moisture interaction, swelling, matrix formation and compatibility with the other components.

Water hyacinth has been proven to be a potential source of cellulose and nanocellulose in previous studies [14]. The present study further shows that the functional properties of the material are good, as compared to commercial cellulose, and that a coherent biopolymer sheet can be obtained from the separated cellulose.

P. crassipes cellulose and carboxymethyl cellulose are also used to produce biodegradable bioplastic film [17]. The present study builds upon this by highlighting the optimization of cellulose extraction and physicochemical characterization prior to the fabrication of the sheets to correlate extraction efficiency, properties of extracted cellulose and material formation.

Table 2. Comparative analysis between extracted cellulose (Water hyacinth) and commercial cellulose

Functional property	Extracted Cellulose (Water hyacinth)	Commercial cellulose
Bulked Density (g/mL)	0.41 ± 0.3	0.192 ± 0.01
Packed Density (g/mL)	0.83± 0.56	0.265 ± 0.07
Hydrated Density (g/mL)	2.42 ± 0.02	1.67 ± 0.36
Water Retention Capacity (WRC) (g/g)	2.34 ± 0.16	1.93 ± 0.82
Oil Retention Capacity (ORC) (g/g)	3.77 ± 0.02	3.17 ± 0.15
Emulsifying activity (EA)	57.05 ± 0.46	56.05 ± 0.06

The morphology changes of the treated cellulose are consistent with previous studies carried out on cellulose nanofibres obtained from the water-hyacinth [15], [16], [18]. The objective of comparative cellulose extraction and structural characterization of cellulose followed by its sheeting process has been explored in the present investigation while aforementioned studies have been focussed to investigate the fibre isolation and processing for material applications. Observed separation of fibres allows the morphological modification of original biomass. But this is not enough to confirm nanofibre production. To make a direct

comparison of the morphology with previously reported nanocellulose materials quantitative measurements of fibre dimensions would be required.

3.3 Characterization of extracted cellulose

3.3.1. FTIR analysis

The FTIR spectra of the untreated and treated water hyacinth samples were analysed to assess the changes in the chemical structures during the alkali treatment and bleaching [Figure 3]. The O–H stretching bands were found at 3335-3400 cm^{-1} while the C–H stretching was found at about 2901 cm^{-1}. The bands in the range 1789-1643 cm-1 were primarily due to absorbed water molecules and a lower intensity was seen in the sample. The vibrations of CH_2 groups appeared between 1158 and 1145 cm^{-1} and the bands of 1100 to 1050 cm^{-1} were assigned to vibrations of C–C and C–O–C. The presence of cellulose associated C–O–C and C–O vibrations was supported by prominent peak at 1104.7 cm^{-1} along with bands at 1017 and 1055 cm^{-1}.

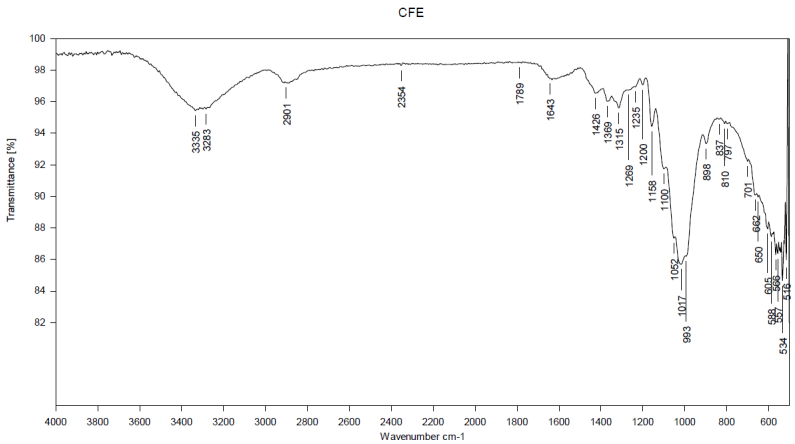

Fig 3. FTIR analysis of water hyacinth fiber (WHF)

The progressive changes in the spectrum after chemical treatment suggest progressive removal of non-cellulosic components and enrichment of cellulose. This is similar to the previous water-hyacinth cellulose research done in which the lignin–hemicellulose matrix was first disrupted through chemical pretreatment before cellulization [10]. The results from the FTIR were verified with the results from the SEM and the XRD analysis, which further supports the results of cellulose enrichment.

3.3.2. SEM analysis of extracted cellulose

The chemical treatment resulted in significant changes in the fibre morphology, as seen in SEM [Figure 4a & b]. The untreated material had larger fibre bundles associated with lignocellulosic material and treatment resulted in dissociation of fibre bundles and formation of fine irregularly shaped fibres. Following treatment, there was a decrease in cementing material between the longitudinal fibres.

The observed fibre separation is similar to that which was reported in chemically treated water hyacinth cellulose and nanocellulose [10], [14]. Treatment with chemicals and fibrillation has been proven to be effective to release cellulose fibres from lignocellulosic matrix [10]. Furthermore, nanofibrillated cellulose from water hyacinth can be easily produced by mechanical homogenization, which is also reported [14]. The present study, however, demonstrates that the chemical treatment may be used to obtain the cellulose in a form of adequate purification and structural modification to process it into sheets using the selected processing steps without the need for high speed homogenization for nanofibrillation.

3.3.3. SEM analysis of the biopolymer sheet

The biopolymer sheet surface at the magnifications viewed was generally smooth and shiny with some irregularities [Figure 4c & d]. In some places, the cellulose fibres were fairly uniformly distributed and areas with lignocellulosic material were observed. The cellulose bundles extracted from materials were turned to be more continuous as observed in fabricated structures during the fabrication process.

The ability to form a coherent sheet indicates that extracted cellulose could be used to produce a useful biomaterial. This has also been demonstrated in previous studies, where they showed that the cellulose formed by *P. crassipes* is biodegradable to form

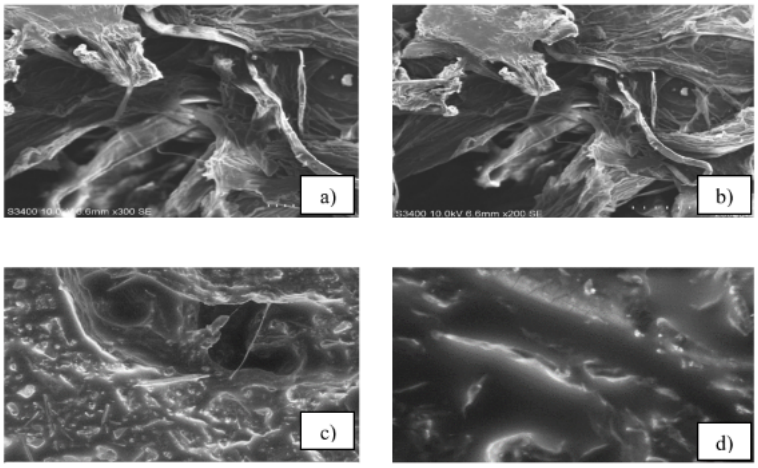

Fig 4. a) SEM image at 100μm ; b) SEM image at 200μm; c) SEM image at 20μm ; d) SEM image at 20μm

a film[17]. The present study complements this work by successfully proving the formation of sheets from the use of cellulose obtained by the optimized extraction method and associating the obtained material with the physicochemical properties of the extracted cellulose. Surface irregularities may be further reduced and mechanical and barrier properties of the sheet may be further enhanced by optimizing the purification of fibres, fibre dispersion and binder / plasticizer composition.

The fabrication results can be applied to the previous studies that have been performed on preparation of water-hyacinth cellulose and carboxymethyl cellulose based biodegradable films[19]. In the current research, both comparative evaluation of the extraction methods, and functional, structural and thermal characterization before the formation of the sheets were carried out. It is its approach of connecting cellulose recovery and characterization of material to the successful formation of a continuous sheet. A quantitative assessment of tensile strength, elongation at break, water-vapour permeability, water resistance and biodegradability, however, is needed to make a direct comparison of the performance of the materials. The results of the present invention, therefore, merely show the feasibility of the sheet making but do not prove it to be the best method of making films reported in the past.

3.3.4. XRD analysis

The XRD pattern showed a major diffraction peak at 22.455° (2θ), together with additional peaks at 11.3756°, 19.0336° and 33.6920° [Figure 5a]. The extracted cellulose showed a high crystallinity index, 87.79%, which means that the cellulose fraction is highly crystalline.

High crystallinity in the present study suggests that the treatment with the chemical was capable of good removal of the amorphous lignin and hemicellulose. The same enhancement of structure has been reported for cellulose and nanocellulose obtained from water-hyacinth[10],[14]. The amorphous material that may be located in the vicinity of the cellulose microfibrils is removed during chemical pretreatment, resulting in a higher relative amount of crystalline cellulose.

The crystallinity value of 87.79% is also significant as the crystalline cellulose regions can play a role in providing structure rigidity and thermal resistance. Based on the XRD result therefore, the optimized treatment was able to enrich the cellulose fraction which was further corroborated by the observations carried out using the FTIR and SEM.

3.3.5. Thermogravimetric analysis

From the TGA result, it is found that the extracted cellulose has good degradation stage [Figure 5b]. The weight loss predominantly occurred in the initial stage of the process between 287.7°C and the major weight loss stage between 350.5°C and 505.4°C (weight loss of 60.53%). The later degradation stage (slower degradation) took place up to around 789°C yielding an additional weight loss of 10.36% and leaving a residual of 20.32%.

The cellulosic fibre extracted is thermally stable as shown by the major degradation region. The thermal behaviour is similar to the one that is generally reported for cellulose rich lignocellulosic materials[14]. It has two high values of crystallinity (87.79%) and thermal resistance, which are suitable for processing to cellulose base materials.

However, the relatively high final residue could suggest that there are thermally stable inorganic or carbonaceous components present. They might be able to contribute more if more ash and elemental/compositional analyses was done.

3.3.6. DSC analysis

A DSC thermogram showed the presence of thermal transitions at the temperature range of 0 – 400 °C (Figure 5c). The peak was exothermic at 30 °C with the transition endothermic at 320 °C. There were no significant further thermal transitions above this temperature. The thermal transition at the temperature of 320°C confirms the observation made by TGA of major thermal transitions of the extracted cellulose in relatively high temperatures. The thermal characterization of water hyacinth, cellulose and nanocellulose from water hyacinth, and other lignocellulosics, have been reported[17]. The results obtained from the agreement between TGA and DSC, which also indicates the thermal stability of the extracted cellulose, confirm the outstanding thermal stability of the extracted cellulose.

3.3.7. Fabrication of biopolymer sheets

The isolated cellulose was successfully transformed to biopolymer sheets [Figure 6 a & b] which shows the possibility of using P. crassipes as a renewable feedstock for biodegradable material production.

Relevance of this application in the light of the previous research where it has been proved that the cellulose and carboxymethyl cellulose derived from water-hyacinth can be used for biodegradable bioplastic films[17]. The present work further follows this research trend by showing an integrated process that starts with comparative extraction followed by functional evaluation, FTIR, SEM, XRD, TGA and DSC characterization before the fabrication of the cellulose-based sheet.

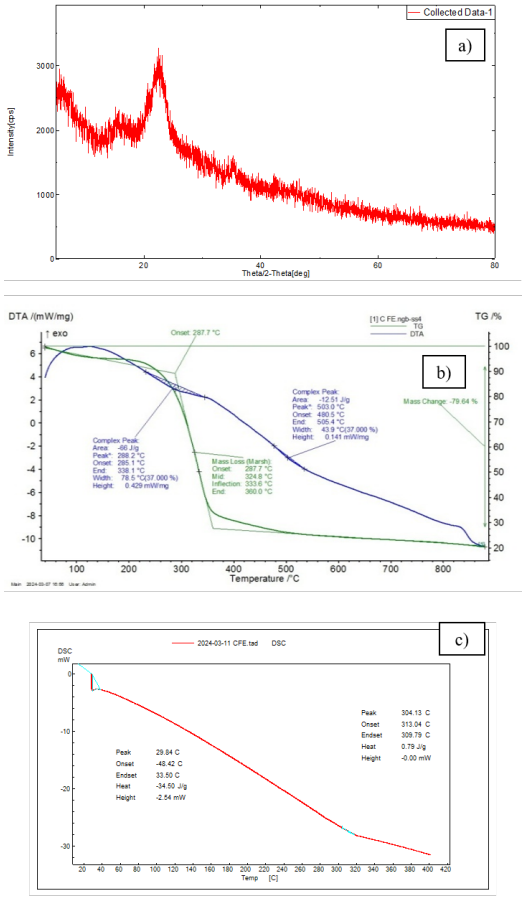

Fig 5. a) XRD spectrum of extracted cellulose from water hyacinth; b) DTA-TGA thermal analysis; c) DSC spectrum of extracted cellulose from water hyacinth

3.4 Comparative significance and novelty of the present study

Water hyacinth is an aquatic plant which is harmful to fresh water environments[20],[21]. Its potential for cellulose nanofibre production, nanofibrillated cellulose[15],[16],[18],[19],[22] and biodegradable films have been previously studies, as well as its potential for biochar production for dye removal[23]. These studies have shown that water hyacinth can be used for various materials and environmental applications.

In this work, three different extraction methods for cellulose are compared, studied by functional evaluation, FTIR, SEM, XRD and TGA-DSC characterization, and used for the fabrication of biopolymer-sheet. Method I produced the highest amount of cellulose ($35.52 \pm 0.24\%$) which is 48% higher than Method II and 344% higher than Method III. The water and oil absorption capacity of the extracted cellulose were higher than the commercial cellulose, the crystallinity index was 87.79%, and the thermal degradation region with a significant percentage of mass loss (60.53%) was between 350.5 and 505.4°C.

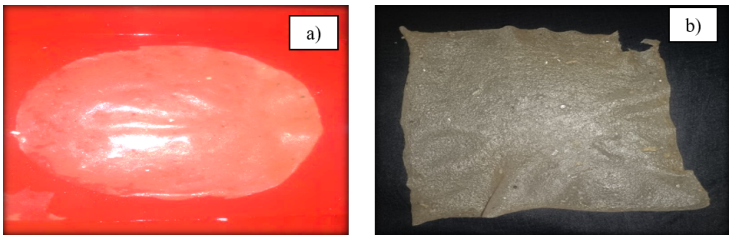

Fig 6. a) Circle shaped biopolymer sheet; b) Square shaped biopolymer sheet

The novelty is not necessarily in the process of cellulose extraction or fabrication of the film, but it is the incorporation of the biomass-to-biomaterial approach. While successful sheet formation shows material development potential, there is need for further studies to determine cellulose purity, mechanical properties, barrier properties, biodegradability, process reproducibility and economic feasibility. These investigations will help to assess the potential of water hyacinth as a sustainable raw material for cellulose-based materials.

4 Conclusion

The present study showed that Method I ($35.52 \pm 0.24\%$ cellulose) was found to be the most effective method among the three extraction methods assessed for the extraction of cellulose from *P. crassipes*. The cellulose obtained showed an improvement in its water and oil absorption capacity compared to the commercial cellulose, crystallinity index of 87.79 % and thermal decomposition in the range of 60.53 % to 65.00 % of its mass. The feasibility of the conversion of recovered cellulose to a useful material was demonstrated in the successful fabrication of the sheets.

The main contribution is the combination of comparative extraction, function evaluation, physico-chemical characterization and sheet production in a single workflow. Cellulose purity, as well as mechanical and barrier properties, biodegradability, process economy should be investigated in further research. The results laid a basis for future valorization of invasive aquatic biomass in the development of materials based on cellulose in the context of a circular bioeconomy.

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6 Author Contributions

Dr. Rathna Kumari A. K. conceived the research idea, designed the study, planned the methodology, and supervised the research throughout the project. **Dr. Sheena E. V.**, provided academic guidance, assisted in data interpretation, and critically reviewed the manuscript. **Udhaya Sri Nivetha S.**, under the guidance of **Dr. Rathna Kumari A. K.**, performed the experimental work, collected the data, and contributed to manuscript preparation. All authors read, revised, and approved the final manuscript.

7 Data Availability Statement

The data generated and analysed during the current study are included within this published article. Additional data are available from the corresponding author upon reasonable request.

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