ISOLATION AND QUANTIFICATION OF CELLULOSE FROM VARIOUS FOOD-GRADE MACROALGAL SPECIES

JANANI MUTHUKUMAR and RAMALINGAM CHIDAMBARAM

Instrumental and Food Analysis Laboratory, School of Biosciences and Technology, Vellore Institute of Technology, Vellore 632014, Tamilnadu, India
✉ *Corresponding author: R. Chidambaram, cramalingam@vit.ac.in*

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Cellulose has become one of the most popular natural materials for food packaging. It is an ideal alternative for eco-friendly packaging since it is biodegradable. Cellulose content was determined in various food grade seaweed samples from Mandapam, Tamilnadu, such as red (*Kappaphycus alvarezii*, *Gracilaria edulis* and *Gelidiella acerosa*) and brown seaweeds (*Sargassum wightii* and *Turbinaria ornate*). In the present work, each sample was subjected to various procedures for yielding an efficient amount of cellulose, such as two-step isolation, solvent, mechanical, repeated acid-base treatment, and holocellulose methods. The yield was found to be highest for the mechanical method, which involved minimal requirement of chemicals, whereas the other techniques resulted in a comparatively lower cellulose proportion because of severe chemical treatment. ATR-FTIR and SEM examination revealed the functional groups and morphology of the isolated cellulose. This is the first study to compare possible cellulose-containing seaweed groups and validate them using ATR-FTIR analysis.

Keywords: seaweed, cellulose, isolation, yield, ATR-FTIR, functional groups

INTRODUCTION

In recent times, environmentally friendly and feasible materials are becoming progressively prevalent in the production of a variety of high value-added products with minimal global consequences.[1] Cellulose is a major structural constituent of plants and a superabundant natural polymer made up of several repeated sugar molecules, β-(1-4)- linked D-glucose units joined with each other in a manner that prevents disintegration. It is suitable for a broad range of applications owing to its low mass, non-toxic nature, tensile stability, hydrophilicity, hygroscopic nature, biocompatibility, and renewability. The robust structure of this biodegradable polymer and its derivatives allows it to be employed for a variety of commercial uses in pharmaceuticals, cosmetics, food industries, construction supplies, paper products, textiles, propellants, and the production of alternative energy sources, such as biofuel, *etc*.[2]

Cellulose can be obtained from various sources using a wide range of both chemical and mechanical techniques. Plants, but also residual wastes from plants, such as maize stalk, rice husks, other cereal husks, soybean, sugar cane, sunflower, castor beanstalks, *etc*., serve as the primary source of cellulose. Due to the sheer growing demand for cellulose and its derivatives, it is vital to explore more cellulose sources using a flexible and adaptive recovery process. The exploitation of lignocellulosic biomass-based materials has numerous advantages over conventional petroleum-based materials, being more cost-effective, eco-friendly and less energy-consuming.[3]

Macroalgal biomass and seaweeds, in particular, are acquiring a lot of attention as potential cellulose sources since they are widely dispersed and fast-growing, requiring little maintenance as they do not need soil, agricultural inputs, fertilizers, or freshwater, rendering them more appealing as cellulose sources, compared to other plant sources.[4] They are commonly used to extract hydrocolloids. However, significant progress has been made in the development of novel biomass processing methods, allowing for the efficient recovery of cellulose from residual biomass, as well as the separation of minerals, enzymes, and hydrogels. Seaweed cell walls majorly contain cellulose, along with several

other macromolecules, such as xyloglucan, mannose, galactose, algin, agarose, carrageenan, and rhamnose-uric acids.

Cellulose can be extracted in large quantities from all three types of seaweeds, such as red, brown, and green. The two significant factors that determine the cellulose yield from algal biomass are the environment and growth period.[5] The provided environmental conditions result in cellulose with unique physicochemical and mechanical properties. Depending on the species, fully grown seaweed can produce up to 34% (w/w) of cellulose. The green seaweed yielded 1.5-34% (w/w) cellulose, according to *Liu et al*.[6] The cellulose yields of brown and red seaweeds, which are high in carbohydrates, ranged from 2.2-10.2% (w/w) and 0.85-18% (w/w), respectively.

For cellulose isolation, sophisticated compound-specific separation techniques must be applied to break the lignin matrix and remove other non-targeted plant components. The most prevalent and successful multiple-step cellulose extraction technique is a combination of both chemical (pretreatments, alkalization, acid treatment, oxidative bleaching) and mechanical processes (sonication, homogenization). The pretreatment is the primary step for cellulose extraction. It is generally conducted to eliminate lignin and a significant amount of hemicelluloses from biomass. The cellulose polymer has a diversified structure, comprising amorphous and crystalline regions. The amorphous portions are easily accessible in any polar solution. Pretreatment procedures, on the other hand, allow the extraction of crystalline parts. The solvent pretreatment removes cellular components as it quickly penetrates the cell wall, increasing the hydrolysis of lignin and parts of holocellulose, which improves cellulose bioavailability. The alkalization process turns crystalline cellulose into alkali cellulose by disrupting the hydrogen bonds in the inter-crystalline arrangement of the cellulose structure, which can be easily depolymerized for further chemical treatments.[6]

Compared to other plant sources, algal cellulose is more easily available and recovered using simple procedures, as algae completely lack or have lower contents of strongly adhesive constituents, such as hemicelluloses and lignin, which promote firm binding of cellulose microfibrils and restrict their use, while providing mechanical properties to the extracellular matrix.[4] In this research, two brown seaweed species (*Sargassum wightii* and *Turbinaria ornate*) and three red seaweed species (*Gracilaria edulis*, *Gelidiella acerosa* and *Kappaphycus alvarezii*) were explored for isolating cellulose using five different extraction methods that included specific pretreatment and purification processes. Acid and alkali treatments were performed with HCl and NaOH for disrupting the polymeric bonds, to convert the complex structure of the cell wall into simpler forms. The pretreatment was followed by two fold bleaching procedures, using NaClO, KOH, and H_2O_2, to remove all untargeted components and generate highly refined cellulosic fibers. Mechanical treatment method was also employed to destabilize the seaweed primary structure and release the cellulose. Then, FTIR was used to identify the distinct functional groups of obtained celluloses. This non-destructive approach allows the qualitative and quantitative content of biomass in the mid-IR region. It indicates molecular fragments, the presence or absence of specific functional groups, and further information about fibre structure.[7]

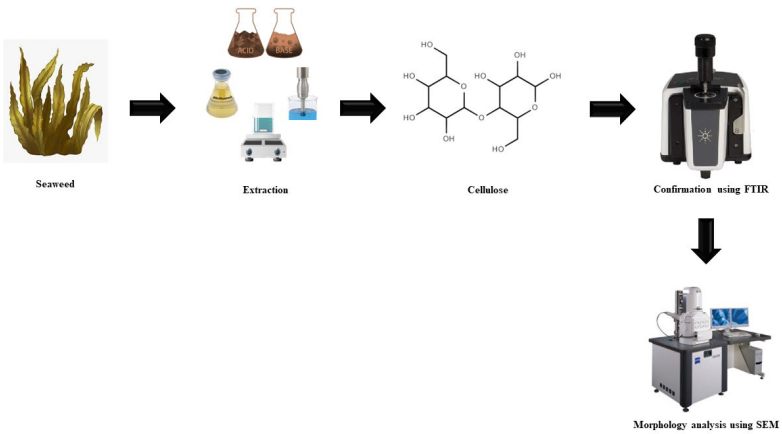

Figure 1: Workflow of the study

Then, morphological analysis using scanning electron microscopy (SEM) was also performed. The workflow of the study is presented in Figure 1.

EXPERIMENTAL

Materials

Brown seaweed (*Sargassum wightii* and *Turbinaria ornata*) and red seaweed (*Gracilaria edulis*, *Gelidiella acerosa* and *Kappaphycus alvarezii*) were collected from the coastal area of Ramanathapuram in Tamil Nadu, India (9 0 50' 43.45" N 780 29' 01.93" E 111 m). Analytical grade chemicals, such as hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium hypochlorite (NaClO), hydrogen peroxide (H_2O_2), glacial acetic acid (CH_3COOH), methanol (CH_3OH), and calcium hydroxide ($Ca(OH)_2$) were purchased from Himedia, India.

Pre-processing of seaweed for extraction

The samples were thoroughly washed several times with tap water to remove unwanted debris before soaking for 24 h in 1 liter of tap water containing 30 mL of 33% HCl for softening the texture. The soaked seaweeds were filtered and rinsed thoroughly with tap water to remove HCl residue. The washed seaweeds were sun-dried for three to four days until they turned brittle. The seaweeds were then pulverized in a mixer and stored in a desiccator for extraction.

Extraction of cellulose

Following seaweed powder extraction, the solid residues were recovered after centrifugation, and cellulose was isolated from them.

In this study, five different cellulose isolation procedures were carried out. Following a recent study by Hansol Doh *et al*.,[12] the first method involved depolymerizing cellulose using acid and alkali, followed by a two-step bleaching process using KOH, NaClO, and H_2O_2. The second method was the holocellulose method using the procedure described by Lakshmi *et al*.,[13] where the carbohydrate fraction of the biomass was isolated by the bleaching process using NaClO, followed by alkali treatment using NaOH to isolate the cellulose alone. The third approach was the solvent pretreatment method, in which the biomass was pretreated with methanol for 24 hours at room temperature before being subjected to bleaching and acid treatment, as suggested by Moohan *et al*.[14] The next procedure involved a unique pretreatment method recommended by Xiao *et al*.,[15] where the water soaked biomass was subjected to ultrasonic treatment to enhance the cellulose extraction efficiency. It was followed by alkalisation, neutralisation and lyophilisation to obtain powdered cellulose. Repetitive base-acid treatment (BABAB) entails the repeated treatment of biomass with alkali and acid using NaOH and HCl, followed by bleaching and sonication processes as reported by Jonjaroen *et al*.[16]

The yield percentage of the isolated cellulose was calculated using the formula:

$$\text{Yield}\% = \frac{W_2}{W_1} * 100 \quad (1)$$

where W_1 is the weight of the raw seaweed powder, and W_2 is the weight of the freeze-dried cellulose.

Characterization of functional groups using ATR-FTIR spectroscopy

The lyophilized cellulose samples were analyzed using an Agilent Cary 630 ATR-FTIR spectrometer, in the frequency range of 4000 to 400 cm^{-1} and at a resolution of 4 cm^{-1}. The spectra were collected in three runs, using 18 scans for each cellulose sample, to identify the compounds and the background. The obtained spectra were processed with Agilent Resolution Pro software, and the generated peak was analyzed to characterize the isolated cellulose.

Morphological analysis

The surface of dried cellulose powder was observed under a ZEISS (EVO18) scanning electron microscope (SEM). The samples were mounted on carbon tape, fixed on aluminium plates and coated with an ultrathin gold coating using a sputter coater. For detecting the microstructure and surface morphology of cellulose, images were obtained in a high vacuum environment at 10 kV with x6K magnification.

RESULTS AND DISCUSSION

In this work, several cellulose isolation methods (two-step isolation, solvent, mechanical, repeated acid-base treatment, and holocellulose method) were investigated for extracting cellulose from other non-targeted elements of five seaweed species – brown seaweeds *Sargassum wightii* and *Turbinaria ornata*, and red seaweeds *Gracilaria edulis*, *Gelidiella acerosa* and *Kappaphycus alvarezii*. The raw material was pretreated with HCl before being subjected to the extraction procedure. Also, all the obtained cellulose samples were freeze-dried. The cellulose samples were subjected to the ATR-FTIR spectroscopic investigation and SEM morphological analysis. Table 1 shows the yield % of the cellulose extracted from each of the species studied. The experiments were performed in triplicates and the results were reported as mean ± standard deviation, using OriginPro 2023 software.

Yield assessment of isolated cellulose

In the two-step isolation procedure, acid-base treatments were followed by a two fold bleaching process. *Gracilaria edulis* had the highest

cellulose output – of 55.4 ± 0.26% in this approach, while *Kappaphycus alvarezii* had the lowest yield – of 4.53 ± 0.40%. The output of the other species was moderate.

In the holocellulose process, all the non-carbohydrate components were eliminated by the treatment with NaClO, and holocellulose was isolated and alkalized for simple cellulose extraction. As a result, *Gelidiella acerosa* produced the highest yield of cellulose (60.63 ± 0.50%), while *Kappaphycus alvarezii* – produced the least (12.13 ± 0.32%).

The solvent method employs CH_3OH to remove cellular components as it quickly penetrates the cell wall, accelerating the hydrolysis of lignin and some of the holocellulose. The use of the solvent thus improves the availability of cellulose. As a result of this treatment, *Turbinaria ornata* yielded 60.57 ± 0.40% cellulose and *Kappaphycus alvarezii* – only 11.53 ± 0.44% cellulose, the two species representing the highest and the lowest cellulose yields.

The ultrasonication technique was employed in this study to destabilize the seaweed's primary structure, allowing it to release the target component *i.e.*, cellulose, due to intense mechanical shear stress incurred by acoustic waves. As ultrasonication is not sufficient as the only treatment to extract cellulose, it was combined with alkali treatment and bleaching. The highest and lowest yields of cellulose obtained by this technique are 74.6% for *Gracilaria edulis* and 29.16% for *Kappaphycus alvarezii*.

Multistep acid-base treatments (BABAB) were performed to fragment the lignin matrix for better compound-specific isolation, followed by bleaching and ultrasonic disruption processes. However, as a result of this procedure, very low cellulose yields were obtained: the highest being of 7.19 ± 0.07% recorded for *Turbinaria ornata*, and the lowest – of 0.38 ± 0.03%, for *Kappaphycus alvarezii*.

Table 1
Yield percentage of isolated seaweed cellulose using five different extraction procedures

Seaweed species	Extraction method Yield (%)				
	Two-step isolation	Holocellulose	Solvent	Ultrasound assisted	BABAB
Sargassum wightii	26.32 ± 0.16	41.23 ± 0.07	40.62 ± 0.08	66.79 ± 0.05	3.11 ± 0.12
Gracilaria edulis	55.1 ± 0.26	24.15 ± 0.22	12.47 ± 0.08	74.58 ± 0.34	3.80 ± 0.07
Turbinaria ornata	33.21 ± 0.21	43.66 ± 0.13	60.57 ± 0.40	60.2 ± 0.30	7.19 ± 0.07
Kappaphycus alvarezii	4.53 ± 0.40	12.13 ± 0.32	11.53 ± 0.44	29.24 ± 0.20	0.38 ± 0.03
Gelidiella acerosa	29.34 ± 0.21	60.63 ± 0.50	25.71 ± 0.14	41.52 ± 0.03	3.10 ± 0.11

FTIR analysis of cellulose samples

The FTIR analysis was performed for the celluloses isolated from the five different species of seaweeds used in this investigation: *Sargassum wightii*, *Turbinaria ornata*, *Gracilaria edulis*, *Gelidiella acerosa* and *Kappaphycus alvarezii*. Figure 2 (a-f) presents the spectra recorded for the celluloses extracted from each of the seaweed species by each of the five extraction methods investigated. The peaks of the spectra were assigned to the corresponding functional groups based on previous literature and the assignments are listed in Table 2. The OH groups were identified in the spectra of the celluloses isolated from all the seaweed species in the region of 3300 cm^{-1}, and their presence explains the hydrophilic nature of cellulose fibers. The stretching vibration of the CH group was correlated with the band between 2800 and 2920 cm^{-1}. The peaks observed at 2921 cm^{-1} in isolated cellulose spectra were assigned to the asymmetrical stretching of CH_2 and CH. The band between 1410 and 1420 cm^{-1} was found to be indicative of CH bending, whereas the band at 1434 cm^{-1} confirms CH_2 symmetric bending in cellulose. After the chemical treatments, the absence of bands at 621 and 1609 cm^{-1} in extracted celluloses confirm the elimination of non-cellulosic components. The

band between 1150 and 1162 cm^{-1} was assigned to C-O-C stretching of the cellulose glycosylic bond.

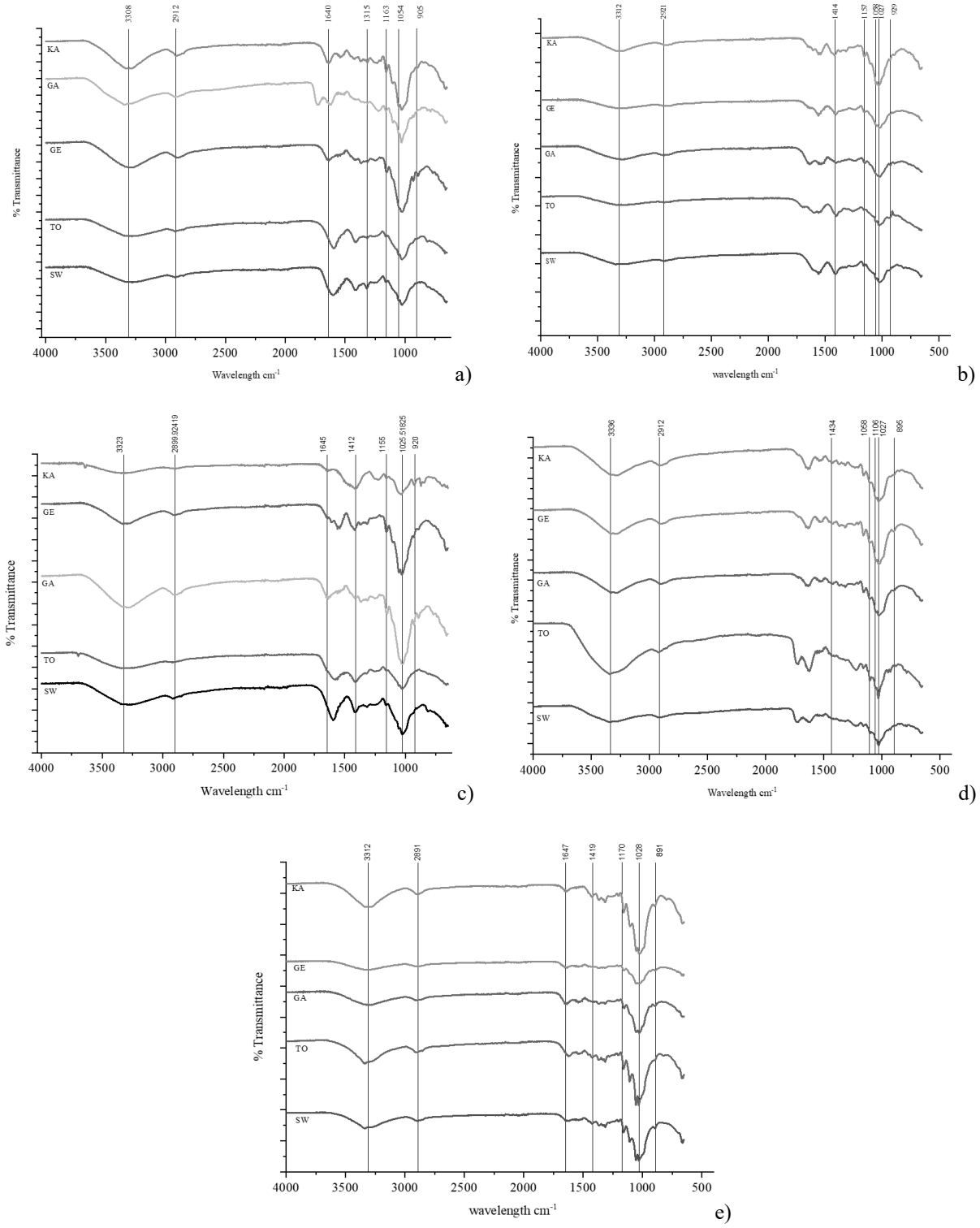

Figure 2: ATR-FTIR spectra of extracted celluloses by (a) two-step isolation, (b) holocellulose, (c) ultrasound assisted, (d) solvent and (e) BABAB methods; (SW – *Sargassum wightii*, TO – *Turbinaria ornate*, GE – *Gracilaria edulis*, GA – *Gelidiella acerosa*, KA – *Kappaphycus alvarezii*)

Table 2
Assignment of FTIR absorption peaks according to literature

No	Wavenumber (cm^{-1})	Functional groups	Reference
1	3308, 3312, 3323, 3336	-OH stretching	17
2	2921	Asymmetrical stretching of $-CH_2$ and -CH	18
3	2891, 2896, 2904, 2912	-CH stretching	19
4	1633, 1640, 1645, 1647	-C=O, -N=O; absorbed -OH stretching vibration	20
5	1434	$-CH_2$ symmetric bending	21
6	1412, 1414, 1417, 1419, 1420, 1423	-CH bending of cellulose	22
7	1315	$-CH_2$ tip vibration	23
8	1155, 1157, 1160, 1161, 1162, 1163	-C-O-C- stretch of glycosylic bond of cellulose	24
9	1054, 1058	-CF stretch of cellulose	25
10	1032, 1036, 1037	-S=O; sulfone, alkane	26
11	1021, 1027, 1028, 1029	-CO stretching vibration	27
12	846, 890, 895, 905, 920, 929	-CH vibration; glycosidic link between sugar units; glycosidic $4C_1$ ring confirmation	28

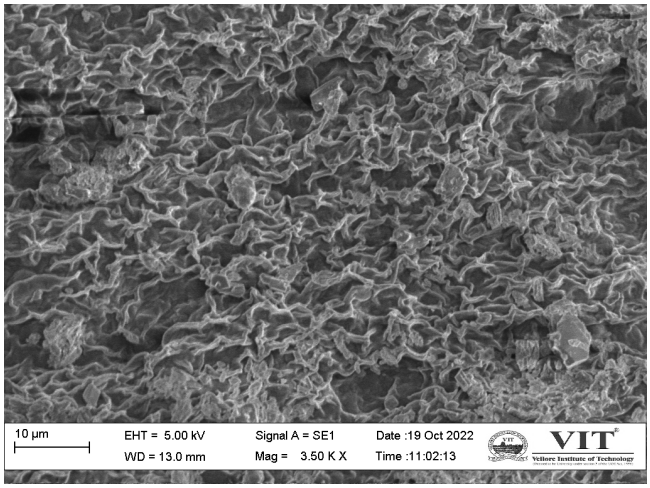

Figure 3: SEM micrograph of cellulose extracted by the ultrasound assisted approach

The analyzed spectra of the obtained celluloses confirmed that cellulose was successfully isolated from the five different seaweed species used in the study. Comparative analysis of the spectra revealed that all of them showed the characteristic peaks of cellulose, and the results were in agreement with previous findings.

Morphology analysis

Since the findings of the yield assessment of all five extraction methods revealed that ultrasonication combined with alkali treatment and bleaching was the most efficient treatment, producing the highest yield of cellulose, the cellulose sample obtained by this approach was subjected to SEM. The morphology of the cellulose obtained by the ultrasound assisted method can be seen in the SEM micrograph shown in Figure 3. Fibrils with thread-like structure and uneven distribution may be noticed, indicating that the treatment causes fibrillation, and fibres break down into smaller fragments, which may be useful for certain applications where enhanced surface area is important.

CONCLUSION

In this work, cellulose was effectively recovered and quantified from five distinct seaweed species, using five different extraction techniques. The ultrasound assisted method yielded very high amounts of cellulose from *Sargassum wighitti*, while the holocellulose and solvent methods gave only modest yields from this species. High yields were also obtained by

the solvent and ultrasound assisted methods from *Turbinaria ornata*, whereas the holocellulose approach produced a fair yield. The holocellulose method showed the maximum yield from *Gelidiella acerosa*, followed by the ultrasound method, which showed a moderate yield. The yield produced from *Gracialaria edulis* employing the ultrasound approach was found to be almost equivalent to the source material quantity, which indicated maximum efficiency obtained by this method, whereas the same species produced a higher-moderate level of yield using the two-step approach. Comparing the yields obtained from *Kappaphycus alvazerii* by all the extraction procedures investigated, it may be concluded that it contains a very low content of cellulose, which makes it unsuitable for cellulose extraction.

FTIR analysis of the cellulose samples confirmed the successful isolation of cellulose by all five extraction methods. However, by comparing the yields obtained by these methods, it is clear that the ultrasound assisted method (combined with alkali treatment and bleaching) provided the highest cellulose extraction efficiency. Another advantage of this technique is that it uses a minimal amount of chemicals. The obtained celluloses can be further investigated to find its suitability for specific applications, for example as filler in the development of biodegradable food packaging.

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