



Isolation and Quantification of Cellulose from Various Food Grade Macroalgal Species and its Characterization using ATR-FTIR Spectroscopy

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Abstract: 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. Macroalgae, commonly known as seaweeds, are a well-known natural source of polysaccharides among marine resources. Cellulose content was determined in various food grade seaweed samples from Mandapam, Tamil Nadu 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 and holocellulose methods which involved minimal requirement of chemicals whereas the other techniques resulted in comparatively less cellulose proportion due to severe chemical treatment. The isolated cellulose was characterized using Attenuated Total Reflectance- Fourier transform infrared spectra (ATR-FTIR), which indicated their respective functional group. 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.

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1. Introduction

In recent times, imperishable, environment-friendly, and feasible materials are becoming progressively prevalent in the production of a variety of high-

value-added products with minimal global consequences (Li *et al.* 2021). Cellulose is the superabundant natural polymer made up of several repeated sugar molecules, β -(1-4)- linked D-glucose units linked with each other in a manner that prevents its disintegration. They are a structural constituent of plants, made up of a significant polymer structure, like synthetic materials suitable for a broad range of applications owing to their low mass, nontoxic nature, tensile stability, hydrophilicity, hygroscopic nature, biocompatibility, and renewability (Liu *et al.* 2021). These biodegradable polymers and their derivatives are incapable of being ingested by living creatures, but their robust structure allows them to be deployed for a variety of commercial uses in pharmaceuticals, cosmetics, food industries, construction supplies, paper products, cotton, linen, jute, rayon for textile industries, propellants, and the production of alternative energy sources such as biofuel, etc (Liu *et al.* 2021).

Cellulose can be obtained from various sources using a wide range of both chemical and biological techniques. The non-fibrous form of cellulose can be found in a variety of green raw materials in different compositions, which includes grass, fruits, veggies, legumes, whole grain products, nuts, seeds, and plants. As a result, residual wastes from plants such as maize, rice husks, cereals, kernel, soybean, sugar cane, sunflower, castor beanstalks, and others serve as a primary source of cellulose. The non-plant forms of cellulose can be found in fossil woods, peat, lignite, sapperite (mineral cellulose), and tunicin (animal cellulose) (Pennells *et al.* 2020). 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. Macroalgal biomass or seaweeds is acquiring a lot of attention as a potential cellulose source since they are widely dispersed and fast-growing biomass which requires little maintenance as they do not require soil, agricultural inputs, fertilizers, or freshwater, rendering them to be more appealing to cellulose source than conventional resources (Zanchetta *et al.* 2020). They are commonly used to extract hydrocolloids. However, significant progress has been made in the development of novel biomass processing methods that allow 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 rhamnase-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 its growth period (Benselfelt *et al.* 2018). The provided

environmental conditions result in cellulose with unique physicochemical and mechanical properties. Depending on the species, a 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.* 2019). The cellulose yields of brown and red seaweeds, which are high in carbohydrates, ranged from 2.2 to 10.2% (w/w) and 0.85 to 18% (w/w), respectively. For significant cellulose production, the cellulose industries primarily use lignocellulosic biomass, such as wood, cotton, flax, hemp, and jute. The exploitation of lignocellulosic biomass-based materials has numerous advantages over traditional sources, such as being more cost-effective, eco-friendly, and low energy consumption (Bhatia *et al.* 2020). The algal cellulose is easily available and recovered using simple procedures, as they lack strongly adhesive constituents like hemicellulose and lignin, which promote firm binding of cellulose microfibrils and restrict their use while providing mechanical properties to the extracellular matrix (Zanchetta *et al.* 2021).

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 (pre-treatments, alkalization, acid treatment, oxidative bleaching) and mechanical processes (Sonication, homogenization). Pre-treatment is the primary step for cellulose extraction. They are generally conducted to eliminate lignin and a significant amount of hemicellulose from the seaweed biomass. The cellulose polymer has a diversified structure, comprising amorphous and crystalline regions. The amorphous portions are easily accessible in any polar solution. Pre-treatment procedures, on the other hand, determine the existence of crystalline parts. Alkalization 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 (Mankar *et al.* 2021). The pre-treatment is followed by bleaching steps for removing the natural pigment to generate highly refined, bleached cellulose, which is further verified using Fourier transform infrared spectra (FTIR).

One of the most prominent ways of identifying the distinct functional groups that make up a molecule is FTIR. The qualitative and quantitative content of biomass in the mid-IR region can be determined using FTIR, which is a non-destructive approach. It indicates molecular fragments, the presence or absence of specific functional groups, and further information about fibre structure.

ATR-FTIR (Attenuated Total Reflectance- Fourier Transform Infrared Spectra) permits incident radiation to be attenuated and infrared spectra to be obtained without aqueous background absorption (Tiernan *et al.* 2020). Long polymeric strands of cellulose obtained from seaweed can be transformed by employing strong acid, into nano ranged materials called Cellulose Nanoparticles (CNP). CNPs are a novel class of cellulose having functional capabilities that differ from native cellulose. CNPs can be categorized into nanocrystals and nanofibers. These CNPs have a larger surface area, aspect ratio, and enhanced thermomechanical properties (Rana *et al.* 2021). Hence, they have emerged as the most enticing and novel materials for various applications such as food packaging, composite materials, filter medium, coatings, medicines, sorbent products, etc.

In this research, diverse brown seaweed species (*Sargassum wightii* and *Turbinaria ornata* and red seaweed species *Gracilaria edulis*, *Gelidiella acerosa*, and *Kappaphycus alvarezii*) were employed to isolate cellulose using five different extraction methods that include specific pre-treatment and purification processes. The current study aims to compare ATR-FTIR investigations of cellulosic fibers from different seaweed sources to identify their functional groups.

2. Materials and Methods

2.1. 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 78 0 29' 01.93" E 111 m). Analytical grade chemicals such as hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium hypochlorite (NaOCl), hydrogen peroxide (H₂O₂), glacial acetic acid (CH₃COOH), Methanol (CH₃OH), and calcium hydroxide (Ca(OH)₂) were procured from Hi-Media, India,

2.2. Pre-Processing of Seaweed for Extraction

The samples were thoroughly washed several times with tap water to remove unwanted debris before soaking for 24 hours in 1 litre of tap water containing 30 ml of 33% HCl for softening its texture. The soaked seaweeds were filtered and rinsed thoroughly with tap water to remove HCl residue. The washed seaweeds were sundried for three to four days till they turned brittle. The

seaweeds were then pulverized in a mixer and stored in a desiccator for extraction (Muthukumar *et al.* 2020).

2.3. Extraction of Cellulose

2.3.1. Two-step isolation

Following recent work by Muthukumar *et al.* (2020), cellulose was extracted. The powdered seaweed sample was immersed in a beaker containing 0.2 M HCl in (1:10) w/v ratio to water for 2 hours at 30°C. The pre-treated colloidal solution was rinsed and centrifuged repeatedly until it was neutralized. The solution was immersed in water (1:60) w/v and maintained at 75°C for 3 hours to reach a pH of 10.5 using 4% NaOH. After stirring, the colloidal suspension was centrifuged for 10 minutes at 15,000 rpm, and the pellet was oven dried for 3 days at 60°C. As a primary step in bleaching. The oven-dried residues were soaked in 10% KOH solution for 3 hours to remove the polysaccharide residual barrier. The samples were treated with 6.5% NaOCl for 2 hours at 75°C after several washing, and the pH was adjusted to 5 using CH₃COOH. The secondary bleaching is performed by adding 30% active H₂O₂ (g/5 ml) to the sample and kept at stirring for 70 minutes at 80°C. The bleached samples were obtained by centrifuging the suspension at 22,000 rpm for 10 minutes which was later subjected to freeze-drying to obtain powdered cellulose.

2.3.2. Holocellulose method

As reported by Lakshmi *et al.* (2017), the cellulose was recovered from seaweed samples. As a pre-treatment step, seaweed was soaked in a beaker containing 500 ml of water with 5% NaOCl solution for 2 hours at 80°C to obtain the holocellulose. The holocellulose was adjusted to pH 7 with 4% NaOH after filtration and subsequent washing. The holocellulose was treated with 40 ml of alkali (17.5% NaOH) followed by the addition of 20 ml of NaOH every 5 minutes until the sample reached a total volume of 100 ml. The mixture was then added with 120 ml of water and left undisturbed for 90 minutes at room temperature. The alkali concentration was reduced by adding 60 ml of 10% CH₃COOH. The cellulose fibres were washed until they were acid-free and then freeze-dried to obtain powdered cellulose.

2.3.3. Solvent method

Seaweed powder was immersed in a beaker containing CH₃OH (1:1) for 24 hours at room temperature as a pre-treatment step proposed by Mohan

et al. (2019). The pre-treated residue was filtered, washed, and mixed with acidified sodium chlorite (pH 2-3) for 3 hours at 60°C with constant agitation, followed by repeated washing and filtering. Subsequently, 30 ml of HCl was added and heated for 5 minutes. The extracted mixture was cooled to room temperature overnight and lyophilized for 24 h using a freeze dryer to obtain powdered cellulose.

2.3.4. Ultra-sonication method

For the extraction of cellulose from the seaweed samples, a unique pre-treatment procedure recommended by Xiao *et al.* (2021) was up taken. Initially, the seaweed powder was soaked in a beaker containing water for 2 hours at 60°C which was further sonicated at 30 kHz with an amplitude of 40% for 40 min. The pre-treated samples were alkalized for 2 hours at 55°C with 6 g of Ca(OH)₂. The samples were then washed and filtered to neutralize them. The resulting cellulose was lyophilized for 24 hours in a freeze dryer to obtain powdered cellulose.

2.3.5. Repetitive base acid (BABAB) treatment method

Seaweed powder was soaked in a beaker containing 4% NaOH solution (1:1) for 24 hours at room temperature. The pre-treated samples were heated with 4% HCl for 1 hour at 75°C and the residue was later washed and filtered. The above two stages were repeated twice, with a filter and a wash in between. The treated sample was bleached using 10% NaOCl along with a few drops of 4% HCl for 2 hours (Jonjaroen *et al.* 2020). It was sonicated for 15 minutes at 30 kHz with a 40% amplitude and then allowed to cool at ambient temperature for 1 hour. Powdered cellulose was obtained by lyophilizing the acquired sample for 24 hours in a freeze drier.

2.4. Characterization of Functional Groups using ATR-FTIR Spectroscopy

The lyophilized cellulose samples were analysed using Agilent Carry 630 ATR-FTIR spectroscopy with a frequency range of 4000 to 400 cm⁻¹ and a resolution of 4 cm⁻¹. The experiment was done in triplicates, using 18 scans on both sides of each cellulose sample to identify the compound and the background. The obtained spectra were processed with Agilent resolution pro software based on the functional group of spectra, and the generated peak was analysed to characterize the isolated cellulose. Fig. 1 (a-e) illustrates the extracted cellulose samples before and after freeze drying.

3. Statistical Analysis

All the experiments were performed in triplicates. Significant differences between the values were estimated using the ANOVA.

4. Results and Discussions

Each of the isolation methods was used to extract the cellulose from the other non-targeted elements of the seaweeds such as lignin, pectic, etc., followed by the ATR-FTIR spectroscopic investigation. Pre-treatments, alkalization, acid treatment, oxidative bleaching, sonication, homogenization, and other techniques were employed to extract the cellulose. All the extracted cellulose samples were freeze-dried. Table 1 shows the yield% of freeze-dried cellulose from *Sargassum wightii*, *Turbinaria ornata*, *Gracilaria edulis*, *Gelidiella acerosa*, and *Kappaphycus alvarezii*. The yield percentage of the isolated cellulose was calculated using the formula $Yield\% = \frac{W_2}{W_1} * 100$, where W_1 is the weight of the raw seaweed powder source, and W_2 is the weight of the freeze-dried cellulose.

4.1. Yield Assessment of Isolated Cellulose

4.1.1. Two-step isolation

Acid-base treatments were performed with HCl and NaOH for disrupting the polymeric bonds using the depolymerization process to convert the complex structure of the cell wall into simpler forms. The residual barriers for isolating the cellulose were eliminated from the depolymerized sample using KOH treatment. It is followed by a two-fold bleaching procedure using NaOCl and H_2O_2 was used to remove all elements except cellulose, such as lignin, hemicellulose, surface contaminants, and pigments. This technique causes the cellulosic fibers to decolorize and improve their adhesion. *Gracilaria edulis* had a higher cellulose output of 55.4% in this approach, while *Kappaphycus alvarezii* had a lower yield of 4.6%. The output of the other species was moderate.

4.1.2. Holocellulose method

In this method, all the carbohydrate fractions are extracted by NaOCl followed by the isolation of cellulose by the alkalization process. The powdered seaweed samples were exposed to NaOCl at high temperatures, resulting in the removal of all non-carbohydrate components and the isolation of holocellulose. The holocellulose was treated with NaOH, where the OH^- ions interfere with

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

Seaweeds	Methodologies														
	Two-step isolation			Holocellulose			Solvent			Mechanical			BABAB		
	W1 (g)	W2 (g)	Yield (%)	W1 (g)	W2 (g)	Yield (%)	W1 (g)	W2 (g)	Yield (%)	W1 (g)	W2 (g)	Yield (%)	W1 (g)	W2 (g)	Yield (%)
<i>Sargassum wightii</i>	5	1.31	26.2	5	2.06	41.2	5	2.03	40.6	5	3.34	66.8	5	0.15	3
<i>Gracilaria edulis</i>	5	2.77	55.4	5	1.2	24	5	0.62	12.4	5	3.73	74.6	5	0.19	3.8
<i>Turbinaria ornata</i>	5	1.65	33	5	2.19	43.8	5	3.09	60.2	5	3	60	5	0.36	7.2
<i>Kappaphycus alvarezii</i>	5	0.23	4.6	5	0.6	12	5	0.60	11.98	5	1.46	29.16	5	0.02	0.38
<i>Gelidium acerosa</i>	5	1.47	29.4	5	3.04	60.7	5	1.29	25.8	5	2.08	41.52	5	0.15	3

hydrogen linkages between polysaccharides, allowing for simple cellulose extraction. The highest cellulose production was 60.7% in *Gelidiella acerosa*, and the lowest was 12% in *Kappaphycus alvarezii*.

4.1.3. Solvent method

The pre-treatment method employs solvents like CH_3OH to remove cellular components as it quickly penetrates the cell wall. They increase the hydrolysis of lignin and parts of holocellulose, which improves cellulose bioavailability. Since the HCl accelerates the oxidation rate of the bleaching process, acidified NaOCl is utilized as an effective bleaching agent, followed by alkalization and acidification with NaOH and HCl, respectively. *Turbinaria ornata* of 60.2% and *Kappaphycus alvarezii* of 11.98% respectively, had higher and lower cellulose yields.

4.1.4. Mechanical method

The Ultrasonication technique is employed to destabilize seaweed's primary structure and leach out the target component i.e., cellulose using intense mechanical shear stress from acoustic waves. In this study, the combination of ultrasonication, alkalization, and bleaching was performed to obtain higher cellulose yields. The higher and lower yields of cellulose in this technique are 74.6% for *Gracilaria edulis* and 29.16% for *Kappaphycus alvarezii*.

4.1.5. BABAB method

To effectively isolate cellulose and remove other plant elements, the seaweed's lignin matrix must be fragmented. This can be achieved by multistep acid-base treatments for better compound-specific isolation, extended with bleaching and ultrasonic disruption processes. Initially, the pre-treatment of seaweed in alkali solution enhances the elimination of compounds such as alcohols, phenols, and carboxyl groups which dissociate easily at higher pH. The chlorite oxidation process is accelerated by the acidified bleaching agent, and uniform homogenization can be attained using an ultrasonication approach in which untargeted components are discharged into solvents and can be eliminated by washing. *Turbinaria ornata* produced a yield of 7.2%, while *Kappaphycus alvarezii* produced a yield of 0.38%.

4.2. Determination of Functional Groups

ATR-FTIR can be used to determine the chemical composition of a material. The peaks of the spectrum reflect functional groups, which depict the chemical

Table 2: Characterization of the functional group using ATR-FTIR spectroscopy

S.No	Wavenumber (cm ⁻¹)	Functional groups	References
1	3308, 3312, 3323, 3336	-OH Stretch	(Xionget al. 2019)
2	2921	Asymmetrical stretching of -CH ₂ and -CH	(Tarchoun et al. 2019)
3	2891, 2896, 2904, 2912	-CH stretch	(Suciayati et al. 2021)
4	1633, 1640, 1645, 1647	-C=O, -N=O; absorbed -OH stretch vibration	(Bogolitsyn et al. 2020)
5	1434	-CH ₂ Symmetric bending	(Mondal et al. 2021)
6	1412, 1414, 1417, 1419, 1420, 1423	-CH bending of cellulose	(Jmel et al. 2019)
7	1315	-CH ₂ tip vibration	(Benselfelt et al. 2018)
8	1155, 1157, 1160, 1161, 1162, 1163	-C-O-C- stretch of glycosylic bond of cellulose	(Salemand Ismail2020)
9	1054, 1058	-CF stretch of cellulose	(Bhutiya et al. 2020)
10	1032, 1036, 1037	-S=O; sulfone, alkane	(Filik et al. 2012)
11	1021, 1027, 1028, 1029	-CO stretching vibration	(Gao et al. 2018)
12	846,890, 895, 905, 920, 929	-CH rock vibration; glycosidic link between sugar units; glycosidic 4C ₁ ring confirmation	(Salem and Ismail2020)

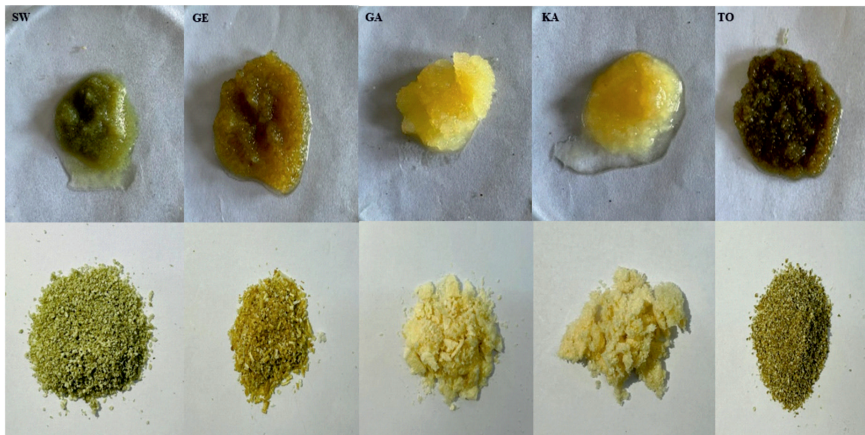


Figure 1 (a)

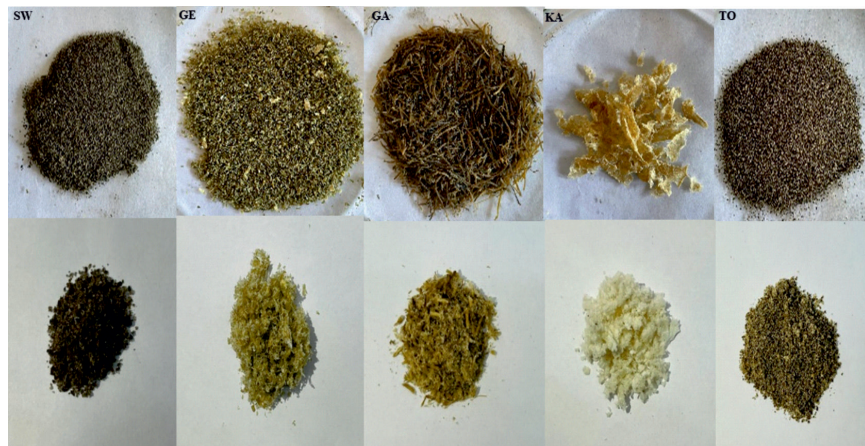


Figure 1 (b)

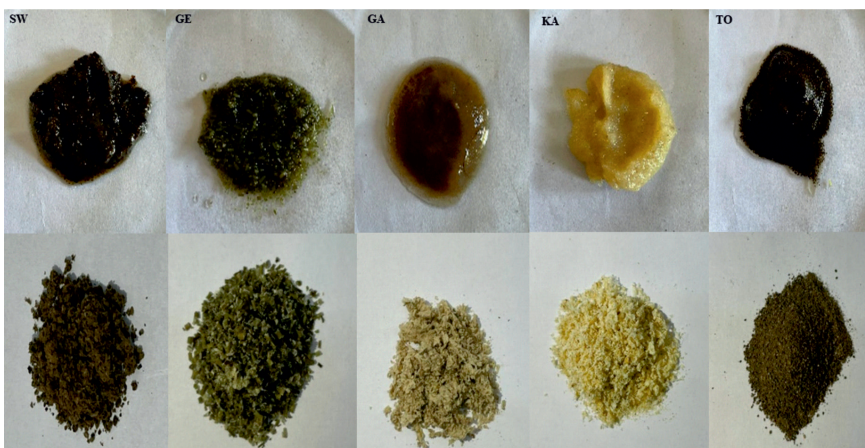


Figure 1 (c)

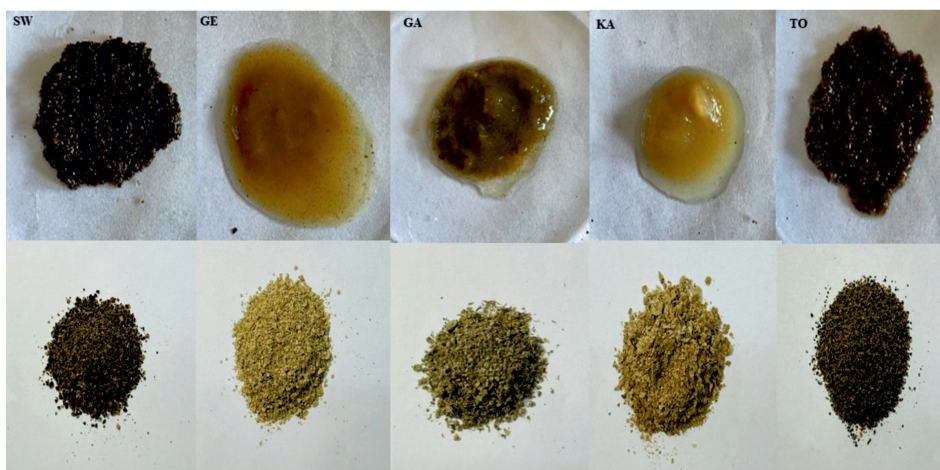


Figure 1 (d)

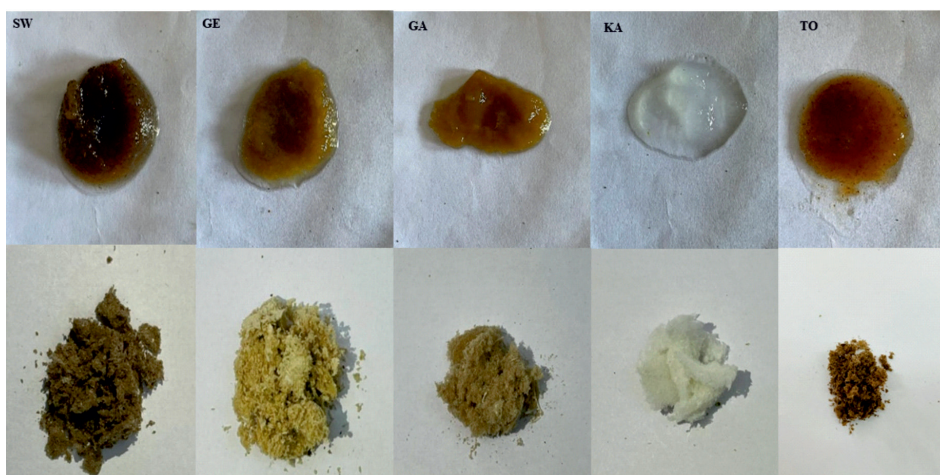


Figure 1 (e)

Figure 1: Extraction of cellulose using (a) two step isolation procedure (b) holocellulose method (c) mechanical method (d) solvent method (e) BABAB method. The non-lyophilized and lyophilized samples are shown above and below, respectively. SW-*Sargassum wightii*; TO- *Turbinaria ornata*; GE- *Gracilaria edulis*; GA- *Gelidiella acerosa*; KA- *Kappaphycus alvarezii*

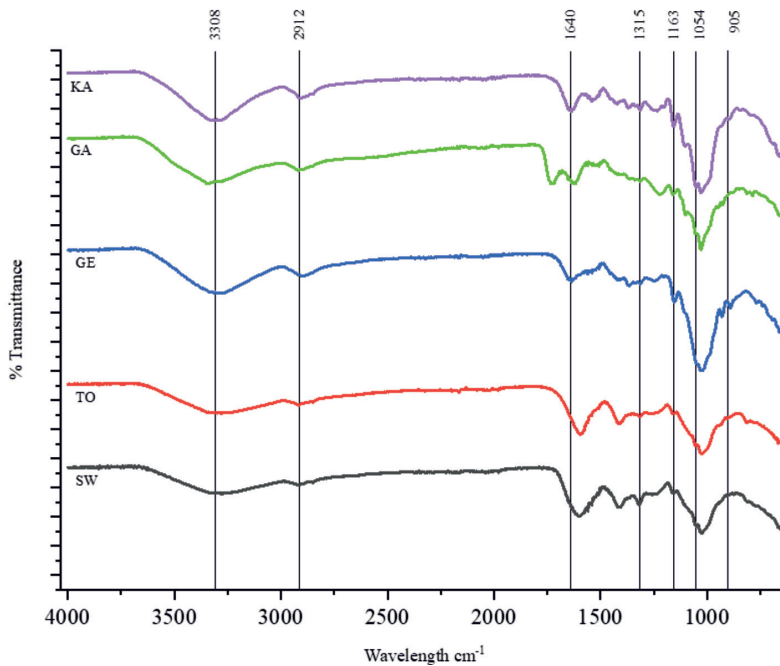


Figure 2 (a)

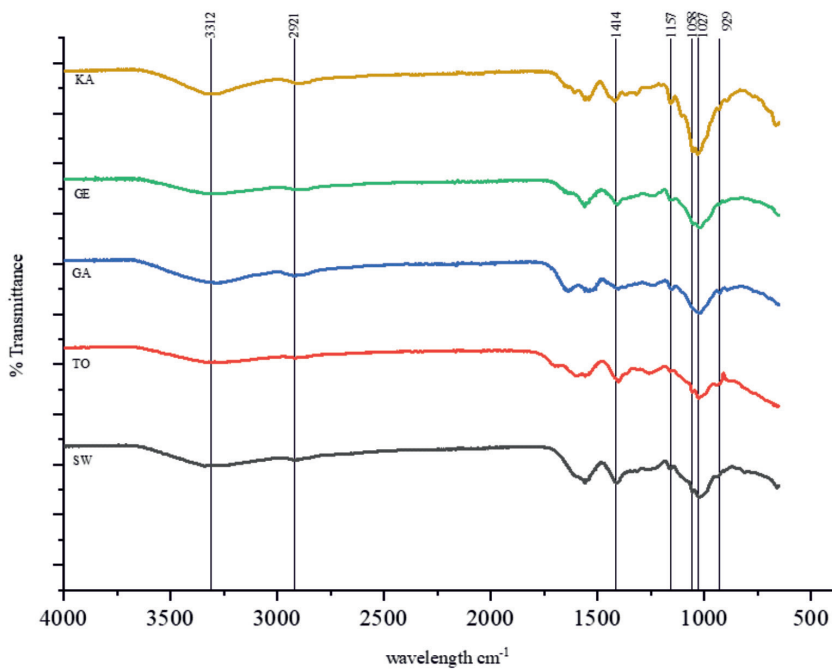


Figure 2 (b)

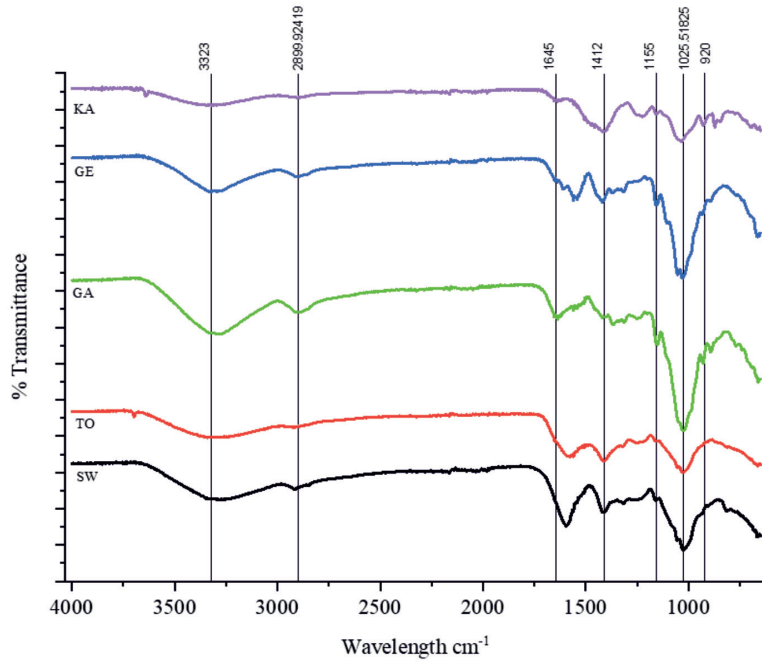


Figure 2 (c)

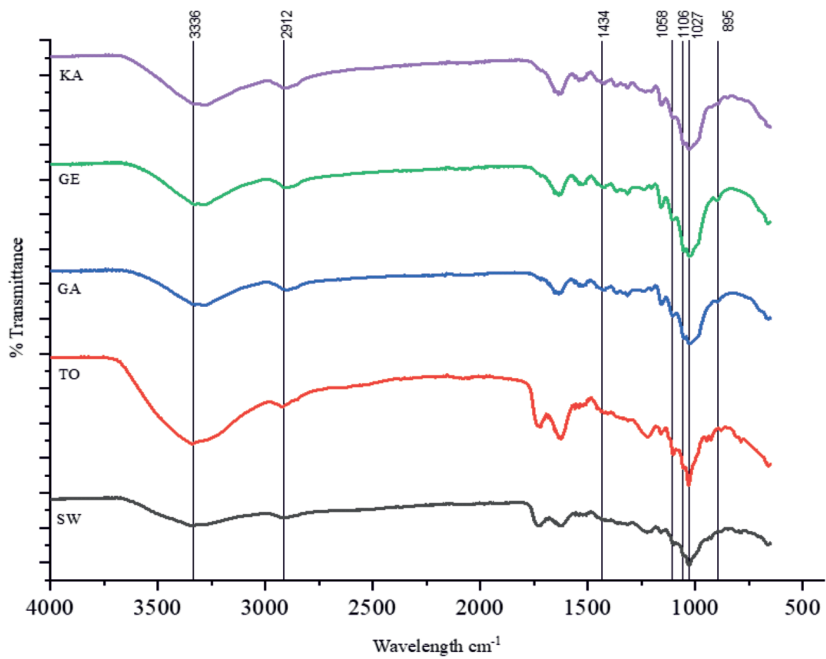


Figure 2 (d)

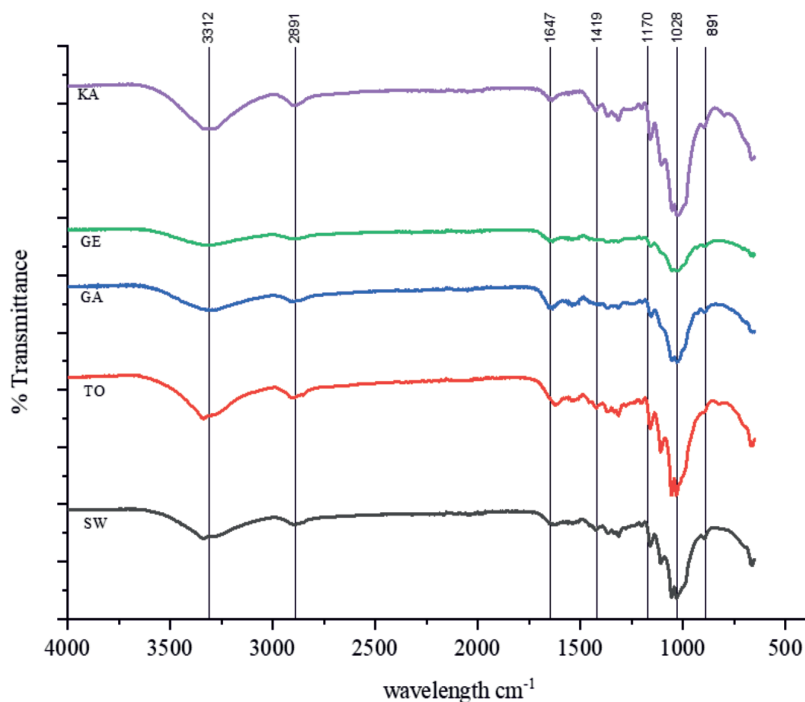


Figure 2 (e)

Figure 2: ATR-FTIR spectra of the extracted cellulose using (a) two step isolation procedure (b) holocellulose method (c) mechanical method (d) solvent method (e) BABAB method

SW- *Sargassum wightii*; TO- *Turbinaria ornata*; GE- *Gracilaria edulis*; GA- *Gelidium acerosa*; KA- *Kappaphycus alvarezii*.

bonding identified in the sample, which was based on a previous study on similar samples. The FTIR analysis was performed for the isolated cellulose from five different species of seaweed. The spectra were obtained using the potassium bromide (KBr) pellet method in the 4000–400 cm^{-1} range. Table 2 lists the functional groups that were identified based on the acquired spectra, and Fig. 2 (a-e) depicts the spectral representation of each seaweed cellulose generated from each process. Due to the hydrophilic tendency of fibers, hydroxyl groups were identified in the spectra of isolated cellulose in all seaweeds in the region of 3300 cm^{-1} . The stretching vibration of the CH group was correlated to a band between 2800 and 2920 cm^{-1} . The spectral bands observed at 2921 cm^{-1} in isolated cellulose were assigned to the asymmetrical stretching of CH_2 and CH, showing cellulose properties. The presence of phenols, carboxyl acids, and aldehydes is indicated by the presence of carbonyl groups ($\text{C}=\text{O}$) in the

band between 1620 and 1650 cm^{-1} . 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 subsequent chemical treatments, the absolute absence of the bands identified at 621 and 1609 cm^{-1} in extracted cellulose substantiated the elimination of non-cellulosic components. The band at 1315 cm^{-1} implied that CH_2 was vibrating at its tip. The C-O-C stretching of the cellulose glycosylic bond was found between 1150 and 1162 cm^{-1} , while the CF stretching was found between 1054 and 1058 cm^{-1} . Peaks in the region between 890 to 930 cm^{-1} suggested the existence of β -glycoside in glucose units. These patterns indicated that cellulose was well exposed throughout the alkali and bleaching treatments. These results confirmed that cellulose was successfully isolated from the five different seaweeds and was in accordance with previous findings.

5. Conclusion

This work presents the results of yield and ATR-FTIR spectroscopic comparative study of cellulose from seaweed samples with reference to the previous investigations. Cellulose was effectively recovered and quantified from five distinct seaweeds using five different extraction techniques. The mechanical method yielded the most cellulose from *Sargassum wighitti*, while holocellulose and solvent methods gave modest yields. The solvent and mechanical methods produced the highest yield for *Turbinaria ornata*, whereas the holocellulose approach produced a fair yield. The holocellulose method showed the maximum yield for *Gelidiella acerosa* followed by the mechanical method which showed moderate yield. The yield produced from *Gracilaria edulis* employing the mechanical approach was found to be almost equivalent to the source quantity, which indicated maximum efficiency from the method, whereas the same sample obtained using the two-step method showed a higher-moderate level of yield. From all extraction procedures, *Kappaphycus alvarezii* contains relatively minimal cellulose, making it unsuitable for cellulose productivity. The spectra obtained from spectroscopic analysis represent the respective functional groups which confirm the successful isolation of cellulose from each extraction method. These can be further subjected to various characterization techniques to study their structural and functional properties for incorporating them into the production of cellulose nanoforms in future research work. They can be used as fillers to improve the mechanical and thermal qualities of biodegradable food packaging.

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