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Cellulose Isolation and Characterization of Green Seaweed *C. Lentillifera* from Halmahera, Indonesia

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Abstract

Caulerpa lentillifera or known as sea grapes is a type of green seaweed which is rich of nutritional components and widespread in the tropical regions in Asia, including Indonesia. Moreover, *C. lentillifera* contains of polysaccharides, such as cellulose which has the potential to various applications. In this study, *C. lentillifera* collected from Halmahera, Indonesia was determined for its chemical compositions (moisture, ash, extractives, hemicellulose, α-cellulose contents) and was extracted to obtain cellulose. Isolation of cellulose from *C. lentillifera* was done by soxhlet extracted using ethanol-benzene solvent to remove extractives, boiling to increase the amount of cellulose extracted, H_2O_2 bleaching to eliminate any remaining pigments and other contaminants, and freeze drying to get coarse powder of cellulose. The moisture content, ash, extractives, hemicellulose, α-cellulose of *C. lentillifera* were 11.94%, 31.62%, 11.53%, 35.57%, and 7.95%, respectively. The yield of cellulose obtained was 31.13% based on seaweed dry weight. FE-SEM (Field Emission-Scanning Electron Microscopy) analysis of *C. lentillifera* showed colonies of diatoms in elliptical shapes. FTIR (Fourier Transform Infrared) measurements indicating cellulose purity after extraction process. X-Ray Diffraction (XRD) analysis resulted some peaks of salt crystals in *C. lentillifera* and cellulose of *C. lentillifera* in amorphous form. After extraction, the crystallinity index of cellulose obtained was 37.3%.

INTRODUCTION

Seaweed is a benthic macroalgae that is plentiful in Indonesia and a source of essential nutrients. It is recorded that Indonesia is the second biggest production in seaweed in the world with the widespread cultivated land. Besides that, seaweed has fast growth, can be harvested all year round, and the cultivation process does not require large areas of land, fertilizers or pesticides (Salem & Ismail, 2021). There are approximately 911 species of seaweed in Indonesia, some with high economic value but only several species are being mass cultivated. Of all the species, one type of seaweed cultivated in Indonesia maritime waters is *Caulerpa lentillifera*.

C. lentillifera, or known as sea grapes is a type of green marine seaweed (*Chlorophyceae*) that is

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widespread mostly in the tropical regions in Asia, including Indonesia (Nguyen et al., 2011; Nofiani et al., 2018) This seaweed spreads to eastern Indonesia, Maluku waters, Bunguran Natuna Islands, and Nusa Tenggara (Razai et al., 2019). *C. lentillifera* grows in shallow seas and has general characteristics in the form of a thallus resembling a stolon, having rhizoids and assimilator with round ramuli resembling grapes. This commodity is mainly utilized in terms of food, medicine, or beauty industry because of the superior nutrition contains in it (Tapotubun et al., 2020). The chemical compositions of seaweed might be varied because of climate and environmental conditions where it was grown. So far, chemical composition of *C. lentillifera* from Halmahera, North Maluku, Indonesia has not been reported yet.

Furthermore, *C. lentillifera* contains polysaccharides as major components on the cell wall (Honwichit et al., 2022). Cellulose as one of polysaccharides in seaweed is potential for various applications, from conventional materials, such as the paper industry, to advanced materials such as bioplastic, composites, biomedical, and drug delivery (Fatriasari et al., 2019). Previous research showed that the cellulose content in *Caulerpa sp.* was 8.7% (Nurjanah et al., 2018). Cellulose is a polysaccharide which is consist of β-D-glucose units linked via (1→4) glycosidic bonds. Cellulose is mainly obtained from terrestrial plants, wood and non-wood sources (Joseph et al., 2023). In plants, cellulose is building the cell walls with hemicellulose and lignin (Fernández-Rodríguez et al., 2018). However, the presence of lignin makes the process of isolating cellulose in plants requires harsh chemical treatment causing slight cellulose degradation (Ververis et al., 2004). Recently, cellulose from seaweed has increased attention due to the absence of lignin in seaweed leading to purer cellulose obtained and also the extraction process under milder condition so that less degraded cellulose (Wahlström et al., 2020). To our knowledge, information about *C. lentillifera* and cellulose extracted from *C. lentillifera* have not been widely discussed in the available literatures. Therefore, this research intends to isolation of cellulose from *C. lentillifera* and characterization of *C. lentillifera* and its cellulose.

MATERIALS AND METHOD

Materials

C. lentillifera was collected from Halmahera, North Maluku, Indonesia. Hydrogen peroxyde 4%, acetone, ethanol-benzene (1:2 v/v), $NaClO_2$ 25%, acetic acid glacial 100%, NaOH 17%, NaOH 8,3%, acetic acid 10% were purchased from Merck Germany, aquades, and RO (*Reverse Osmosis*) water.

Sample Preparation

The collected seaweed was rinsed several times using tap water to remove any dirt in samples. Next, the samples were dried under the sun for 2 days followed by oven at 60°c for 1 day to reduce water content. Dried *C. lentillifera* were grinded with blender to become coarse powder. The sample used for the extraction of cellulose was *C. lentillifera* powder that passes through a 16 mesh sieve.

Cellulose Extraction

The extraction of cellulose from *C. lentillifera* was done using method suggested by Bar-Shai (Bar-Shai et al., 2021) with some modification. *C. lentillifera* powder was soxhlet extracted for 6 hours at temperature of 80°C using ethanol-benzene (1:2 v/v) solvent to remove extractives. Next, the samples were boiled in water with a ratio of sample and water was 1:20 (v/v) at 80°C for 45 minutes to dissolve remaining impurities and increase the amount of insoluble fiber such as cellulose extracted from seaweed. The boiled water was discarded and the samples then purified by 4% H_2O_2 solution with ratio 1:80 (v/v) at 80°C for 5 hours to eliminate any remaining green pigments and other contaminants including hemicellulose. Next, the supernatant was discarded and pulp cellulose was washed by water until neutral. Subsequently, cellulose was freeze-dried to become powder. Procedure of extraction cellulose was shown in Figure 1. Cellulose yield after freeze-drying procedure, was is calculated by Eq. (1).

$$\text{Yield (\%)} = \frac{\text{Weight of cellulose}}{\text{Initial weight of seaweed}} \text{ x } 100\% \qquad (1)$$

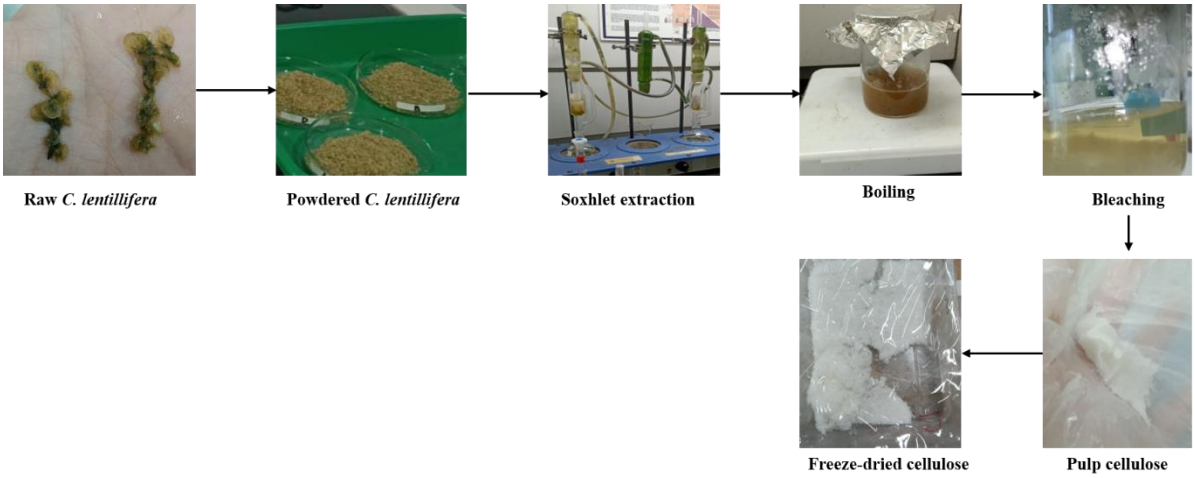

Figure 1. Prosedure of extraction cellulose from *C. lentillifera*

Characterization

FTIR Measurements

Characterization of powder and cellulose of *C. lentillifera* was conducted using the FT-IR ATR (Fourier Transform Infrared *Attenuated Total Reflectance*) method by Spectrum Two Perkin Elmer FTIR in the range of 4,000 cm^{-1} to 400 cm^{-1} to determine their functional groups.

X-Ray Diffraction (XRD) Measurements

XRD test of *C. lentillifera* powder and *C. lentillifera* cellulose was carried out using Shimadzu Scientific Instruments XRD-MaximaX 7000, at a range of 2 theta angle from 10° to 60°. The crystallinity index (X_c) was obtained using the Eq. (2).

$$X_c = \frac{Ac}{Aa + Ac} \times 100\% \quad (1)$$

where A_c is area of all the crystalline peaks, and A_a+A_c represents the total of all the crystalline and amorphous peaks.

Morphological Studies

Field emission-scanning electron microscopy (FE-SEM) was used to see the surface morphology of *C. lentillifera* powder. The test was carried out by using FE-SEM Thermo Scientific Quattro S, which was operating at a voltage of 1 kV at magnifications of 1000x.

Chemical Composition Analysis

In brief, *C. lentillifera* powder heated in the oven at 105°C for 24 hours and 550°C for 6 hours for moisture and ash contents analysis, respectively. Extractives content analysis was carried out using soxhlet method with ethanol-benzene (1:2 v/v) solvent at temperature of 80°C for 6 hours. Holocellulose content analysis was performed by adding 25% $NaClO_2$ and glacial acetic acid into extractive-free sample, then heated at 80˚C. Afterward, the sample was washed with cold water and acetone, then dried to obtain hollocellulose. Then, into the holocellulose sample was added by 17% NaOH and aquades, and reacted for 45 min, then rinsed with 8.3% NaOH and aquades. Next, the sample was added to 10% acetic acid and neutralized to get α-cellulose. The hemicellulose content was obtained from the subtraction of holocellulose content by α-cellulose content.

RESULTS AND DISCUSSION

Extraction of Cellulose

The extraction procedure of cellulose from *C. lentillifera* started with grinding to enhance the surface area for better chemical reaction (Sundari & Ramesh, 2012). The next step was soxhlet extraction using etanol-benzene solution to remove extractive, followed by boiling process to dissolve remining impurities. The boiling process resulted in an increase in insoluble fiber levels such as cellulose (Yuanita, 2010). The sample then purified by using hydrogen peroxide to eliminate any remaining green pigments and other contaminants including hemicellulose, which is more preferred in a biorefinery process from a green chemistry point of view. The resulting cellulose extracted from *C. lentillifera* was clear white with the yield of 31.13%, which is higher than cellulose obtained from other *Caulerpa sp.* up to 22.61% (Nurjanah et al., 2018).

Table 1. Chemical components of *C. lentillifera.*

Chemical composition (%)	***C. lentillifera***	
	Halmahera, Indonesia	**Okinawa, Japan (Rabemanolontsoa & Saka, 2013)**
Moisture content	11.94 ± 0.4	-
Ash	31.62 ± 0.4	50
Extractives	11.53 ± 1.44	5.4
α-cellulose	7.95 ± 1.64	5.6
Hemicellulose	35.57 ± 0.37	20.7

The extracted cellulose from *C. lentillifera* was presented in Figure 2.

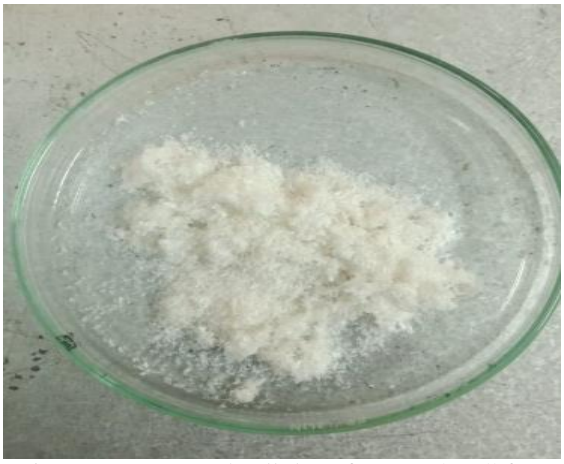

Figure 2. Extracted cellulose from *C. lentillifera*

Chemical Composition of *C. Lentillifera*

The chemical composition of *C. lentillifera* collected from Halmahera, Indonesia is presented in Table 1. The extractives, cellulose, and hemicellulose contents of *C. lentillifera* from Halmahera are higher than of *C. lentillifera* from Japan, except of ash content. The chemical component level differences of *C. lentillifera* might be influenced by their habitat, maturity level, and environmental conditions (Ito & Hori, 1989). The moisture content of *C. lentillifera* from Halmahera after drying process was 11.94±0.4%, whereas the ash content of *C. lentillifera* was 31.62±0,4%. The higher ash content was associated with the higher of mineral elements contained in *C. lentillifera* (Ratana-arporn & Chirapart, 2006). Extractives mostly consist of low molecular compounds soluble in liquids of low polarity such as fats, phenolics, resin acids, and waxes (Pecha & Garcia-Perez, 2020; Rabemanolontsoa & Saka, 2013). In this study, *C. lentillifera* contains 11.53±1,44% of extractives content. Total of α-cellulose and hemicellulose in *C. lentillifera* from Halmahera were 7.95±1,64% and 35.57±0.37% respectively. In seaweed, hemicellulose is the predominant carbohydrate. The majority of the hemicellulosic saccharides in seaweed may be caused by the polysaccharides from *C. lentillifera* were heteropolysaccharides, such as mannose, glucose, galactose, and xylose, which were the main constituents of hemicellulose (polyose) (Honwichit et al., 2022; Konishi et al., 2012; Long et al., 2020).

XRD Analysis of *C. Lentillifera* and Extracted Cellulose

From XRD graphs in Figure 3, there are some peaks with high intensities correspond to various salt crystals presented on the surface of *C. lentillifera* (Bulota & Budtova, 2015; Long et al., 2020). Peaks at 2θ of 27.3°, 31.7°, 45.5°, and 56.6° were confirmed as diffraction peak of halite sodium chlorite (NaCl) crystal (Bao et al., 2017). The XRD patterns of potassium chloride (KCl) contain two main peaks at 28.5° and 40.9° (Ismail et al., 2022).

After going through the removal extractives, boiling, and bleaching process, the *C. lentillifera* cellulose XRD graphs, still showing some peaks of amorphous-like cellulose. The extracted cellulose from *C. lentillifera* displays three peaks at 2θ of 13.5° reflection assigned to the (1 0 1) crystallographic plane, broad peak at 2θ of 18.8° – 19.9° reflection assigned to amorphous region of cellulose, and at 2θ of 22.8° reflection assigned to the (0 0 2) or (2 0 0) crystallographic plane of cellulose I allomorph (Popescu et al., 2011). Cellulose extraction of other green seaweed, *Ulva lactuca* also resulted amorphous-like cellulose. It is concluded that the cellulose microfibrils as cell wall constituents, interwoven with xylan or mixed xylan-glucan polymers (Lahaye et al., 1994). The crystallinity index of cellulose from *C. lentillifera* was 32%. The low crystallinity of extracted cellulose probably because it is a mixtures of cellulose with a xylose-glucose (xyloglucan) polysaccharide, which

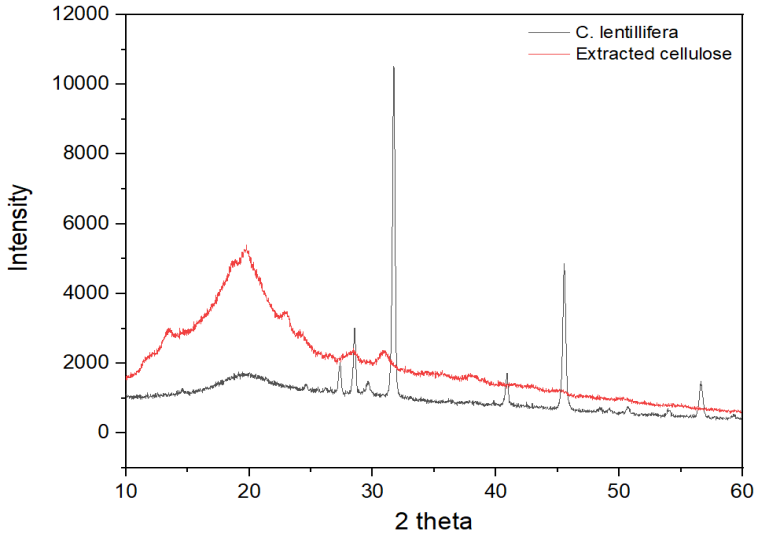

Figure 3. XRD analysis of raw material *C. lentillifera* and extracted cellulose.

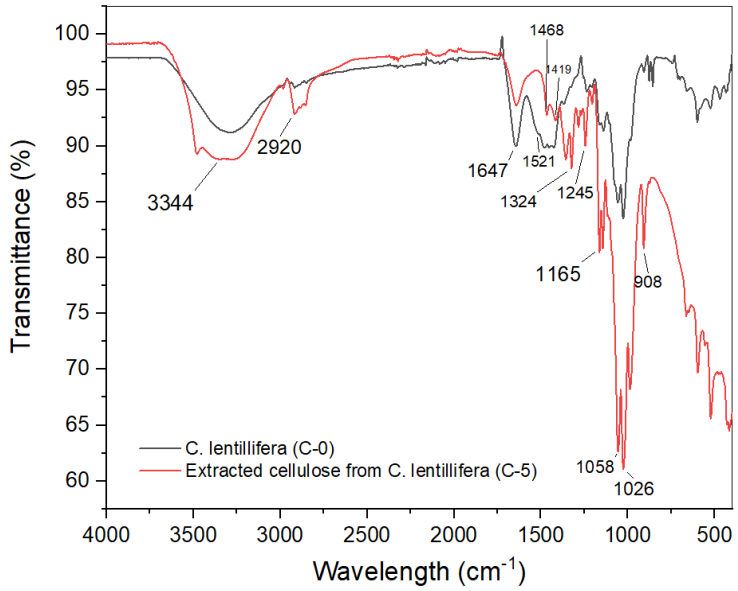

Figure 4. FTIR analysis of raw material *C. lentillifera* and extracted cellulose.

affected formation of cellulose crystals leading to a low crystallinity (Wahlström et al., 2020). Additionally, some seaweeds contain cellulose as a major component of their cell walls, which tends to make them highly crystalline, while others do not have cellulose as a main composition of their cell walls, resulting in low cellulose content and crystallinity in their cell walls (Mihranyan et al., 2004).

FTIR Analysis of *C. Lentillifera* and Extracted Cellulose

The infrared spectrum of *C. lentillifera* and extracted cellulose shown broad absorbance around 3400 – 3300 cm^{-1}, which were associated to the hydroxyl groups stretching (Becerra et al., 2023). Moreover, there was peak at around 2919 cm^{-1} mainly represent to the symmetrical or antisymmetrical stretching of C-H on methyl and methylene from the aliphatic groups on cellulose or hemicellulose (Chaudhari, 2016). The absorbance at 1647-1645 cm^{-1} demonstrated the stretching of the O-H groups as a result of the cellulose structure absorbing water (Arnata et al., 2020). The peak at 1520 cm^{-1} in IR spectra of *C. lentillifera* powder represent the absorption of amide band II (N–H bending coupled with C–N stretching) from proteins (Long et al., 2020). After treatment to obtain cellulose, The peak at 1520 cm^{-1} disappeared, which means that protein was disappear successfully from the cellulose fraction.

Tabel 2. IR spectrum of functional groups of the sample based on the absorption band (cm^{-1}).

C-0 (cm^{-1})	C-5 (cm^{-1})	Group	Reference
3322	3344	O-H stretching	(Becerra et al., 2023)
2920	2920	C-H stretching	(Chaudhari, 2016)
1647	1646	Adsorbed water	(Arnata et al., 2020)
1520	-	Amide band II (N-H bending)	(Long et al., 2020)
-	1419	H–C–H scissor vibration	(Arnata et al., 2020)
-	1324	H–C–H tip vibration	
1165	1165	C–O–C antisymmetric bridge stretching	
907	908	C-O-C stretching	(Joseph et al., 2023)

The increase in peak sharpness after bleaching at around 1419, 1324, 1165, and 908 cm^{-1} owing to increase of cellulose purity levels (Arnata et al., 2020). The peak at 908 cm^{-1} is associated with the cellulosic β-glycosidic linkages (Joseph et al., 2023). FT-IR spectra on *C. lentillifera* powder and extracted cellulose are presented in Figure 4. The typical functional groups with conforming bands are displayed in Table 2.

SEM Analysis of *C. Lentillifera*

Figure 5 shows SEM micrograph of green macroalgae *C. lentillifera*. *C. lentillifera* depicts large number of elliptically shaped diatoms, as if forming a split right in the middle like a coffee bean. Previous study showed similar result of *C. lentillifera* in rich colonization of elliptically shaped diatoms (Khan et al., 2022).

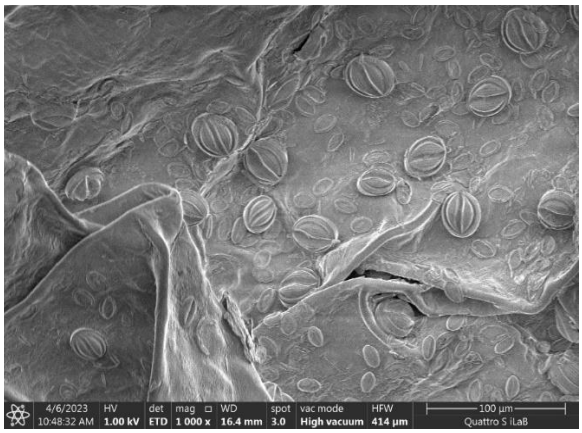

Figure 5. SEM analysis of raw *C. lentillifera*.

CONCLUSION

This study aimed to demonstrate the isolation process of cellulose from *C. lentillifera*, the characterization of *C. lentillifera* and it's cellulose. The chemical analysis of *C. lentillifera* collected from Halmahera, Indonesia show moisture content 11.94%; ash 31.62%; extractives 11.53%; α-cellulose 7.95%, and hemicellulose 35.57%. Extraction of cellulose from *C. lentillifera* has been done by soxhlet extraction using ethanol-benzene solvent, followed by boiling, and bleaching process using hydrogen peroxide. FTIR spectum showed purity of cellulose, which obtained by the disappearance of the protein functional group and the increasing of peak sharpness after final bleaching at around 1419, 1324, 1165, and 908 cm^{-1}. X-ray diffraction graphs of *C. lentillifera* showed the presence of various salt crystal on the surface of *C. lentillifera*. Final bleaching process resulted the amorphous-like cellulose, which might indicate that cellulose fibrils connected with xylan or mixed xylan-glucan polymers. SEM analysis shows rich elliptically shaped diatoms on the surface of *C. lentillifera*.

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