

Research Article

Ecofriendly Isolation of Cellulose from *Eucalyptus lenceolata*: A Novel Approach

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This study reports the extraction of cellulose by means of an environment-friendly multistep procedure involving alkaline treatment and totally chlorine-free bleaching. The multistep process begins with the removal of pectin, cutin, waxes, and other extractives from *Eucalyptus lenceolata* straw, followed by the removal of hemicelluloses and lignin using an alkaline treatment, and lastly by further delignification of the cellulose pulp through a two-step bleaching process, first with the use of hydrogen peroxide/tetraacetylenediamine (TAED) and then with the use of a mixture of acetic and nitric acids. The *Eucalyptus lenceolata* samples were collected from the mountains of the Malakand division of Khyber Pakhtunkhwa, Pakistan and were ground into smaller particles. The pulp resulting from each step was characterized by infrared spectroscopy (ATR-FTIR) to detect structural changes. The purified cellulose was characterized through different analytical techniques such as Fourier transfer infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and scanning electron microscopy (SEM). The isolated cellulose has a high degree of purity and crystallinity (73%) and thermal stability as verified by XRD and TGA, respectively. SEM was used to study the surface morphology of cellulose, indicating that the surface was free from lignin and hemicelluloses due to the chemical treatment. This study indicates that the multistep procedure is quite adequate for the extraction of cellulose.

1. Introduction

The biopolymer of cellulose is abundantly present in the universe. Cellulose composition contains straight chains of D-glucose connected by β -1,4-glycosidic linkage using a high quality form of polymerization of 1×10^3 in native woods. The D-anhydroglucopyranose entity provides OH groups at the positions of carbons 2, 3, and 6, which can undergo the classical reactions identified for primary and secondary alcohols [1]. The crystalline structure of cellulose is a closely packed chain with Van der Waals interactions and numerous forms of intra- and intermolecular hydrogen bonding. The cellulose molecular structure shows properties such as degradability, hydrophilicity, chirality, and wide chemical

inconsistency. Due to its long chain and greater molecular mass, cellulose is insoluble in water [2, 3]. It has been mainly used as a source of paper since the beginning. Besides, cellulose has a wide range of uses in different fields. Cellulose and its derivatives are the focus of the current research work because of the growing demand for it and its importance for bioethanol production [4]. Current research work is aimed at knowing everything about the important use of cellulose obtained from wood, especially synthesizing biofuels which will be a great benefit to our energy requirements and to our need for a green environment. Cellulose fibers are the main constituent of plant cell walls which is a rich source for ethanol production [5]. Cellulose is commonly used as a source of bioethanol. In 2008, the global

bioethanol production remained at more than 41 billion liters. The world's major producers are Brazil (37%), the United States (33%), and Asia (14%). Brazil manufactures bioethanol from sugarcane. Brazil extended its production to more than 16.4 billion liters in 2008 to facilitate the financial investment for nearly 18% of the nation's locomotive petroleum requirements [6].

In recent years, the researches have focused more on the isolation of cellulose from biomass which has a great application in green chemistry. Literature shows that cellulose can be isolated from different sources, including straw of wheat and sugarcane, bagasse, hemp and flax straws, rice straw, rice husk, soybean hulls, jute, banana stems and pineapple leaf fiber, and palm oil residue [7, 8].

Cellulose derivatives contain methyl cellulose, ethyl cellulose, propyl cellulose, etc. The main objective was to isolate all the biochemicals and to find out their applications. In terms of the medical aspect, it can pass through the digestive tract but it cannot be absorbed by the human intestine. The colon can accumulate a huge quantity of water which produces a bulkier and softer substance. Cellulose is also applicable in medicine, where it is used for the treatment of different diseases like hemorrhoids, diverticulosis, diarrhea, and irritable bowel syndrome. Dehydration can be prevented by taking a sufficient amount of cellulose because it has a strong affinity to absorb water [9, 10]. Methyl cellulose is viscous in nature and is used as a lubricant; it is the major component of jellies. Methyl cellulose in lubricating form is used in dry eye treatment. There is less tear secretion by the lachrymal gland and accessory conjunctival glands which is why tear treatment is used [11].

It has many applications in the field of construction. It is used in construction materials for additive performance. By the addition of cellulose to a dehydrated mortar mixture, the properties of workability, water retention, viscosity, and adhesion are increased. It is also used in cement- and gypsum-based industries [12]. Methyl cellulose is utilized in insulating plasters, self-levelling floors, cement panels, tile adhesives, joint and crack fillers, and tile grout [13]. Cellulose is used in glues and binders for the fixation of delicate parts of art and also to clean old glue from books and wallpaper pastes. Methyl cellulose is specially used in culture cell virology to observe virus-related duplication. Normally, cells are grown in a medium which is dissolved in an identical nutrient. Only those cells are grown on the surface, which has a single layer. Moreover, the cells are infected for a short time. The medium, which is prepared from methyl cellulose, is added to the cell by exchanging it with the ordinary liquid medium. Only the infected viruses are able to spread in the infected cells, the membranes of which touch each other. The cells that are close to each other are infected and die [14, 15].

Ethyl cellulose is a derivative form of cellulose in which some of the OH groups on the repeating glucose constituents are changed into ethyl ether groups. The amount of ethyl groups can differ depending on the production conditions. It is mainly used as a thin-film for covering substances. For the preservation of food, ethyl cellulose is used as an emulsifier [16]. Acetate is derived from cellulose by analyzing wood

pulp into purified fluffy white cellulose. It is used as a film base in photography, as a component in some glues, and as a border substance for eyeglasses. It is further used as an artificial fiber and in the production of cigarettes and playing cards. It indicates the properties of selective absorption and adsorption of low levels of organic chemicals. The heat and pressure of plasticizers enable them to simply bond with the acetate of cellulose. It is commonly soluble in many solvents containing acetone and other organic solvents, and it can be further improved to become soluble in another solvent such as water. It is hydrophilic with good liquid passage and exceptional absorption. In textile applications, it delivers comfort and permeability, but its strength also fails when wet. Acetate fibers are used in allergenic treatment. It can simply be composted or incinerated. It can be dyed; however, unusual dyes and pigments are essential since acetate does not accept ordinary dyes used for rayon and cotton. Acetate fibers are resistant to mold and mildew. It is definitely destabilized by strong alkaline solutions and strong oxidizing agents [17].

A nitrocellulose membrane is a sticky membrane used for immobilizing nucleic acid. Because of its nonspecific affinity for amino acids, it is also used for the control of proteins in western blots and atomic force microscopy. Nitrocellulose is usually used as a support in diagnostic tests where antigen-antibody binding occur, such as in pregnancy tests and U-albumin tests. Nitrocellulose lacquer is also used as an aircraft dope, painted onto fabric-covered aircraft to tighten and provide protection to the material [18].

In paper, paper board, and textile industries, it is used for absorbing water or oil. In capillary electrophoresis, it is used as a buffer additive to overcome electroosmotic flow for improved separation [19, 20]. In household applications, it is used in making sunglasses, blouses, buttons, dresses, linings, home furnishings, draperies, wedding and party attires, toothpastes, laxatives, diet pills, detergents, and water-based paints. It is also applicable in nonvolatile eye drops as a lubricant [10, 21].

Eucalyptus is a diverse genus of flowering trees and is one of the tallest flowering plants on the surface of the earth. The motivation for farming eucalyptus established from 1970 to 1985 was for to use it as a fuel wood. More than seven hundred species of eucalyptus are found in Australia, but a few number are present in Indonesia, Philippines, America, Europe, Africa, Middle East, China, and the Indian subcontinent. Moreover, many eucalypts can be planted in the temperate zone. An area of about 92,000,000 hectares is covered with eucalypt forest in Australia. In Pakistan, an area of about 10,000 hectares is covered [22].

2. Material and Methods

Eucalyptus lenceolata was collected from the mountains of the Malakand division of Khyber Pakhtunkhwa, Pakistan. N-hexane (96%) and ethanol were purchased from Scharlau. Sodium hydroxide, hydrogen peroxide (99%), ethylene diamine tetra-acetate, acetic acid (99.8–100%), and nitric acid (65%) were purchased from Sigma-Aldrich. All the chemicals were of high purity and used without further purification.

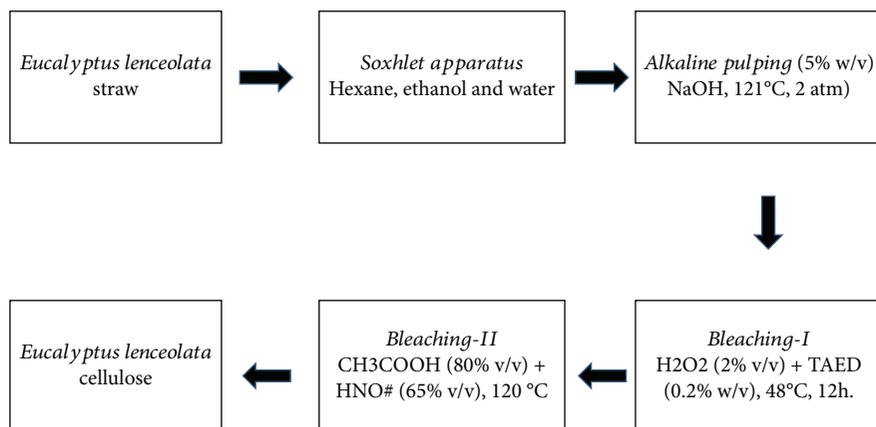


FIGURE 1: Scheme of the isolation steps of cellulose from *Eucalyptus lenceolata*.



FIGURE 2: Sample of crude *Eucalyptus lenceolata* straw (A-0), after the Soxhlet procedure (A-1), after alkaline treatment in an autoclave (A-2), after bleaching-I (A-3), and after bleaching-II (A-4).

2.1. Extraction of Cellulose. Cellulose is a linear polymer which contains crystallites, and thus it has a paracrystalline morphology. The linear cellulose molecules are linked together laterally through hydrogen bonds to form linear bundles, leading to a crystalline structure. During the extraction of cellulose, several changes occur in its structure during the treatment with various chemical substances via pulping [23, 24].

2.1.1. Soxhlet Apparatus. The collected sample was ground into smaller particles with a hammer and passed through an 80-mesh screen. The obtained particles were washed in a Soxhlet apparatus with different solvents in a sequence according to increasing polarity, for example, treatment with n-hexane for 3 hours to remove the lower polar substances, then treatment with ethanol for 3 hours to remove the polar substances, and finally treatment with deionized water to remove the most polar soluble extractives and waxy substances covering the surface of wood particles. The particles were then dried in an oven at 800°C. The overall process of cellulose extraction is systematically shown in Figure 1.

2.1.2. Autoclave. The extractive free straw was treated with an aqueous solution of 5% (w/v) sodium hydroxide (NaOH) for fiber separation and bond breaking using a method projected for soybean hulls [25] but adapted here for *Eucalyptus lenceolata*. The straw suspension was treated in an autoclave (Stermax model 20 EHD) at 121°C and 2 atm of pressure at a 1:100 straw to liquor ratio (g/ml). The period of reaction

TABLE 1: Abbreviations for different cellulosic samples.

Abbreviations	Sample
A-0	Crude <i>Eucalyptus lenceolata</i> straw
A-1	Extract-free <i>Eucalyptus lenceolata</i> straw
A-2	Cellulose pulp after 20 minutes in an autoclave
A-3	Cellulose after bleaching-I
A-4	Cellulose after bleaching-II

was 30 min. The final pulp was filtrated and washed with deionized water until pH was neutral.

2.1.3. Bleaching-I. More polar substances and the remaining hemicelluloses and lignin were removed through bleaching based on a process previously described for wheat straw [26]. The cellulose pulp was treated with a 2% (v/v) hydrogen peroxide (H₂O₂) solution and 0.2% (w/v) ethylene diamine tetra-acetate (EDTA) solution, at pH = 12, for 12 h, at 48°C, under stirring, and at a 1:25 straw to liquor ratio (g/ml). The pulp was filtrated and washed with deionized water until pH become neutral. This step is called bleaching-I.

2.1.4. Bleaching-II. In bleaching-II, the purification of raw cellulose was carried out with an 80% (v/v) CH₃COOH solution at a 1:33 straw to liquor ratio (g/ml) and with a 65% (v/v) of HNO₃ solution at a 1:4 straw to liquor ratio (g/ml), at 120°C, under vigorous mechanical stirring for 30 min [10]. The solid was filtered and washed with ethyl alcohol and double-distilled water until pH was neutral. The samples obtained during each step of the extraction procedure are shown in Figure 2.

The various designations for crude *Eucalyptus lenceolata* straw and cellulosic samples obtained during each step of the extraction procedure are listed in Table 1.

2.2. Characterization. The cellulose was characterized by scanning electron microscopy (SEM) (JSM 5910, JEOL, Japan). XRD was performed using an X-ray diffractometer (Rigaku D/Max-II, Cu Tube, Japan),

2.2.1. Fourier Transfer Infrared Spectroscopy (FTIR). ATR-FTIR spectra were collected with 64 scans and a resolution

TABLE 2: Assignment of infrared adsorption bands of the samples in various wavelengths (cm^{-1}).

A-0	A-1	A-2	A-3	A-4	Assignment
1032	1032	1032	1031	1032	C-O stretching
—	—	—	—	1055	C-O symmetric stretching of primary alcohol
1109	1109	1109	1107	1109	-C-N group absorption
—	—	—	1161	1161	C-O-C antisymmetric bridge stretching
—	—	—	—	1203	OH in-plane bending
—	—	—	1321	1316	CH ₂ deformation
—	—	—	1428	1428	CH ₂ bending
1592	1592	1590	1592	—	C=C stretching of aromatic ring
—	—	—	—	1639	Adsorbed water

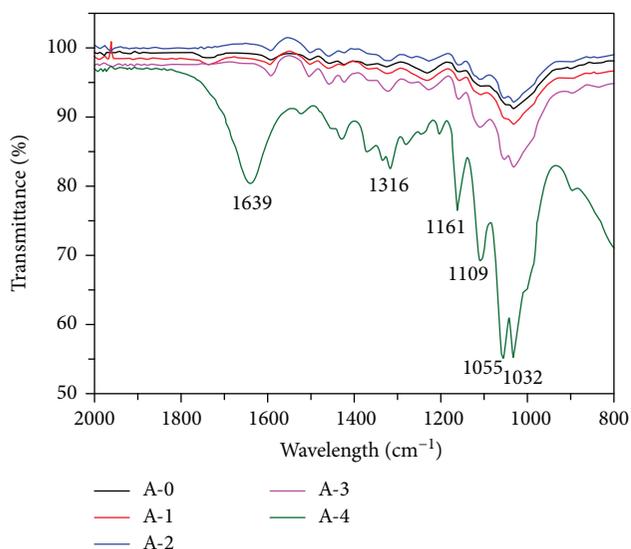


FIGURE 3: FTIR spectra at different stages of cellulose extraction from the wood of *Eucalyptus lenceolata*.

of 2 cm^{-1} in a FTIR-8201 PC Shimadzu Fourier spectrophotometer. Dried maize straw, intermediate samples, and purified celluloses were analyzed by ATR-FTIR in order to make clear structural changes along the various steps of cellulose extraction. The spectra were investigated in the range of $800\text{--}2000 \text{ cm}^{-1}$.

2.2.2. Thermogravimetric Analysis (TGA). TGA measurements were conducted at a heating rate of $10^\circ\text{C min}^{-1}$ under N_2 (50 ml min^{-1}), using a TA Instruments model TGA Q5000 IR. Sample weight was typically kept at 7 mg. The TGA microbalance had a precision of $\pm 0.1 \text{ lg}$.

2.2.3. X-Ray Diffractometry. The XRD experiment was performed using a Siemens D500 diffractometer. Cellulose was scanned in the reflection mode using an incident X-ray of $\text{CuK}\alpha$ with a wavelength of 1.54 \AA at a step width of $0.05^\circ \text{ min}^{-1}$ from $2\theta = 0\text{--}40^\circ$.

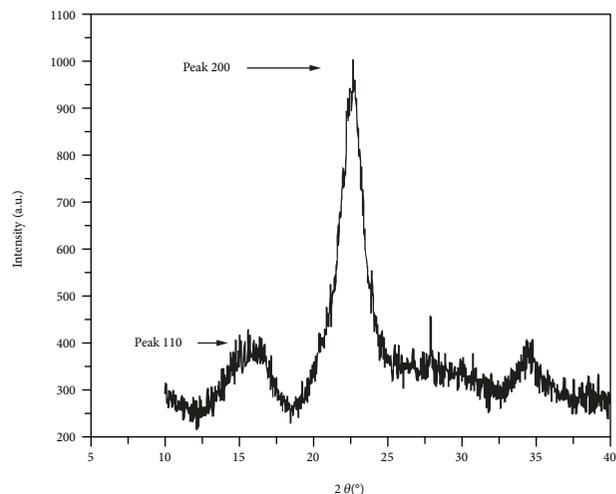


FIGURE 4: XRD spectra of cellulose prepared from the wood of *Eucalyptus lenceolata*.

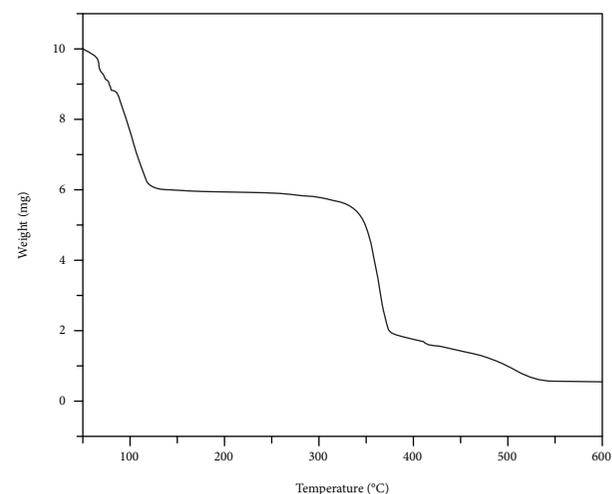


FIGURE 5: TGA spectra of cellulose prepared from the wood of *Eucalyptus lenceolata*.

2.2.4. Scanning Electron Microscopy (SEM). Scanning electron micrographs of extracted cellulose were obtained using a JEOL® microscope JSM-6060 operating at 20 kV. The test specimens were attached to an aluminum stub and sputtered with gold to eliminate the electron charging effects.

3. Result and Discussion

3.1. FTIR Analysis. FTIR analysis was performed for identification of different functional groups on the surface of raw materials and pure cellulose collected from every stage of the isolation [24].

The major constituents of the *Eucalyptus lenceolata* straw are cellulose, hemicellulose and lignin. These ingredients generally present different oxygen-containing functional groups, such as OH, C=O, C-O-C, and C-O-(H) as reported in the literature [26, 27]. The characteristic functional group and the conforming bands for every constituent are shown in Table 2.

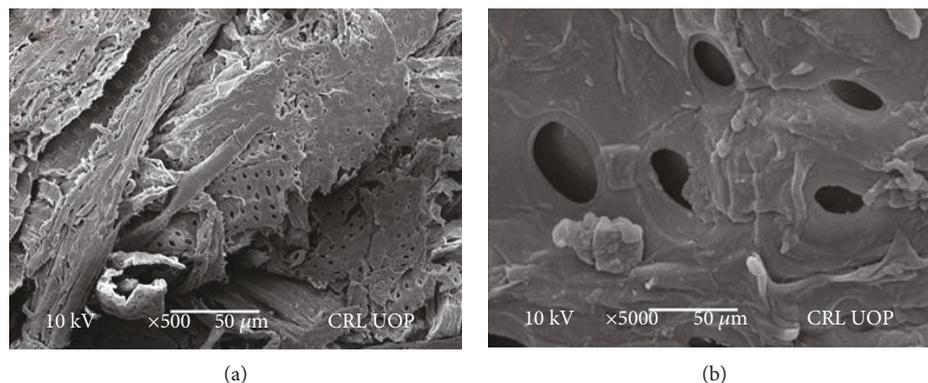


FIGURE 6: SEM micrograph of cellulose prepared from the wood of *Eucalyptus lenceolata* (X500 and X5000 resolutions).

In the spectra, adsorption at 1316 cm^{-1} is associated to aromatic ring vibration which conforms to the appearance of lignin in these samples. The adsorption peak from 1109 to 1161 cm^{-1} shows the presence of C–O–C asymmetric bridge stretching [23, 28]. A strong band appears in pure cellulose after bleaching-II at 1032 – 1055 indicating the presence of a C–O stretching bond in A-4. The rise in spectra at 1639 cm^{-1} can be assigned to H–O–H bending. It also exists in the marketable cellulose. According to the literature, the survival of this spectra indicates the bending mode of adsorbed water. All the samples were totally dried for FTIR analysis as mentioned in the literature. It is too difficult to remove all the water from cellulose because water has a strong interaction with cellulose [29, 30] as shown in Figure 3.

3.2. X-Ray Diffractometry. XRD is an important characterization technique used to determine the crystallinity of a sample. X-rays are used to collect information about the properties of a sample. When the beam of X-rays strikes the surface of target sample, the X-rays are scattered. In scattering, there are possibilities of constructive or destructive interference in a crystalline sample. The crystallinity of cellulose is investigated by X-ray diffraction. According to the literature, Segal's equation is the best method to find the crystallinity of cellulose because it gives the peak height and it does not need any background [30, 31]. Segal's equation is given as follows:

$$X_C = 100 \frac{(I_{200} - I_{AM})}{I_{200}}, \quad (1)$$

where I_{200} is the highest peak which indicates the amorphous and crystalline material, while I_{AM} represents the amorphous part which is in between 200 and 110.

In our research work, the cellulose was prepared from the wood of *Eucalyptus lenceolata*. The highest peak is 1006, and the lowest peak is 269, as shown in Figure 4. Thus, the crystallinity is 73%, which means that it is near the highest crystallinity reported in the literature.

3.3. Thermogravimetric Analysis. Thermogravimetric analysis is the analytical procedure in which the mass of the

sample is observed as a function of temperature or time as the sample specimen is subjected to a controlled temperature program in a controlled atmosphere.

Thermogravimetric analysis is a valuable process for the analysis of organic compounds. It is a particularly useful method to determine the physical and chemical properties of a macromolecule such as cellulose. The TGA graph indicates the loss of mass that took place with respect to time and temperature. The highest temperature in my analyzed data is 600°C . Figure 5 shows the TGA graph of cellulose prepared from the wood of *Eucalyptus lenceolata* indicating the loss of water from the sample that took place at 100°C . Moreover, lignin and hemicellulose were lost at a temperature of 35°C . The TGA curve at 380°C shows the removal of soluble organic extractives. The results of thermogravimetric analysis shows that the data were best fitted according to a literature survey [32, 33].

3.4. Scanning Electron Microscopy. Scanning electron microscopy was used to study the surface morphology of the cellulose extracted from *Eucalyptus lenceolata*. SEM images at different magnifications clearly reveal the removal of pectin, lignin, and hemicellulose particles from the surface [34]. The SEM plates indicate that the surface possesses pores of dissimilar shapes and sizes as represented in Figure 6. As the process gets more advanced, the surface free from lignin and hemicelluloses is more exposed, as can be seen from the SEM images due to the chemical treatment in bleaching-II.

4. Conclusion

Eucalyptus lenceolata is a low-cost, supportable, and renewable source of cellulose, which is critical to modern society's growing environmental concern and demand for energy. Cellulose is extracted from this source using an environment-friendly process, which means that there is no hazardous effect to the environment. The extracted cellulose was analyzed through different analytical techniques like TGA, and the degradation of noncellulosic materials was performed at various temperatures. FTIR determines the removal of different functional groups from cellulose. Through X-ray diffraction, it can be observed that the

sample has a high degree of crystallinity. SEM analysis showed the morphological behavior of the sample surface. It was observed from all the characterization results that the results were best fitted with the literature survey.

Data Availability

All the data supporting the results are shown in the paper and can be requested from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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