Research Article

Isolation and Characterization of Natural Cellulose from *Oxytenanthera abyssinica* (Lowland Ethiopian Bamboo) Using Alkali Peroxide Bleaching Stages Followed by Aqueous Chlorite in Buffer Solution

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Plants are the primary sources of cellulose. This paper is aimed at isolating cellulose from *Oxytenanthera abyssinica* via chemical treatments. The thermal behavior, functional group, chemical composition, crystallinity, and morphology of raw (ROA), dewaxed (DOA), alkali-treated (AOA), and bleached (BOA) fibers were examined. TGA, FTIR, DSC, DTA, XRD, and SEM were used for characterization techniques. The effects of chemical treatments were examined by determining the content of cellulose, hemicellulose, lignin, and ash. The cellulose content in the ROA improved from 49.26 ± 0.13 wt% to 86.01 ± 0.02 wt% due to the removal of noncellulose components using waxing, alkali treatment, and bleaching with alkali peroxide bleaching stages followed by aqueous chlorite in buffer solution. The highest content of cellulose and holocellulose was exhibited in the BOA samples with a yield of 86.01 ± 0.02 wt% and 97.61 ± 0.17 wt%, respectively. ROA had greater hemicellulose (21.31 ± 0.15 wt%), lignin (20.63 ± 0.12 wt%), and ash content (3.30 ± 0.11 wt%) in comparison to AOA and BOA. The XRD data showed a change in crystallinity after each treatment. Because of the high amount of crystalline cellulose, the XRD results revealed that BOA has a higher crystallinity index (CrI) (59.89%) and peak intensity than AOA, DOA, and ROA. The strength of the FTIR peaks increased in the order of ROA, DOA, AOA, and BOA, indicating that pretreatment causes hemicellulose and lignin to be gradually removed from the *Oxytenanthera abyssinica* fiber. The TGA, DTG, DTA, and DSC data also confirmed that BOA has the highest thermal stability due to the high content of cellulose. The SEM analysis showed a morphological change in the surface due to chemical treatment. These results confirmed that through chemical pretreatment, a high amount of cellulose was produced from *Oxytenanthera abyssinica*. Even though *Oxytenanthera abyssinica* is commonly grown in Ethiopia, few studies have been done on it, and no works have been carried out to isolate and characterize cellulose from the plant. Thus, the findings in this work will encourage researchers to use *Oxytenanthera abyssinica* as a source of cellulose for various applications, including the manufacture of cellulose nanocrystals, polymer matrix biofilters, green biocomposite reinforcing agents, and hydrogel synthesis.

1. Introduction

The widespread usage of synthetic or petroleum-based products has raised environmental concerns. Different researchers are producing new, green, sustainable, and multifunctional materials from abundant lignocellulosic biomass [1]. Lignocellulosic biomass is the most abundant source of sustainable carbon material and the most preferred raw
material for manufacturing biochemicals, bioethanol, and biofuels [2]. They are plants or plant-based products that include a variety of natural organic substances [3]. Approximately 85-90% of lignocellulosic biomass contains cellulose, lignin, and hemicellulose [4, 5]. The cellulose biopolymer is the most abundant polymer, found mainly in the cell walls of plants [6]. Globally, its production is predicted to be 1.5 x 10^12 tons per year [7]. In recent years, cellulose has attracted significant interest due to its unique characteristics such as biocompatibility, being environmentally friendly, renewable, and cost-effective, and high abundance in nature [8]. It has various properties, such as good mechanical properties, low density, relatively low cost, good biocompatibility, high thermal stability, durability and strength, and ability to absorb moisture. They are arranged parallel to each other and are joined together with hydrogen bonds [9]. The amount of cellulose in plant species depends on many factors, such as the species type, the plant age, the growing conditions, and the part of the plant [10–12].

Plant cellulose can be extracted using pretreatment procedures. The lignocellulose’s tight structure is disturbed during the pretreatment process, exposing the cellulose fiber to separation [13]. Because pretreatment alters the biomass’s physical, biological, and chemical structure, it is critical to examine the type of pretreatments [14]. Chemical pretreatments are more commonly used than biological or physical pretreatment procedures because they are more effective and improve complex material biodegradation [15]. Hydrogen peroxide (H_2O_2), aqueous ammonia (NH_3H_2O), lime (Ca(OH)_2), potassium hydroxide (KOH), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), acetic acid (CH_3COOH), and sodium hydroxide (NaOH) are common chemicals used in chemical pretreatment procedures for enhancing cellulose structure [16, 17]. Alkali pretreatment is a common chemical treatment that reduces polymerization and disrupts the connection between lignin and other polymers such as cellulose and hemicellulose [18]. It offers a lot of appealing characteristics. It mainly uses nonpolluting noncorrosive chemicals and is carried out in less harsh settings than acid pretreatment. Since they interact predominantly with lignin, they are more effective in removing lignin [19]. The dissolution of hemicellulose and lignin is influenced by four significant parameters such as the base concentration, solid-to-solvent ratio, temperature, and treatment duration [20, 21]. Bleaching is used after alkaline treatment to remove any remaining hemicellulose, lignin, or chromophores using various chemical reagents [22, 23]. Cellulose can be derived from industrial wastes, agricultural residues, wood-based biomass, bacterial metabolism, and even aquatic species [24]. Wood, cotton, hemp, flax, kenaf, bamboo, and jute are the primary sources of cellulose [24].

Ethiopia has Africa’s largest bamboo forest [25]. Ethiopian lowland bamboo called Oxytenanthera abyssinica is an economically and environmentally important bamboo species covering about 85% of the country’s total bamboo area [25, 26]. About 850,000 ha of Oxytenanthera abyssinica is found scattered in the country’s central, southwest, and south parts [27]. Oxytenanthera abyssinica is a high-yielding renewable resource that grows quickly. It is low-cost, fast-growing, and readily available, with physical and mechanical properties similar to wood [28]. The quick growth of Oxytenanthera abyssinica is advantageous in terms of its cellulose sources. However, there is no study on the isolation and characterization of cellulose from the plant; thus, in this study, we aimed to isolate and characterize natural cellulose from the plant through alkali pretreatment and alkali peroxide bleaching stages followed by aqueous chloride in buffer solution.

2. Materials and Methods

2.1. Material. The chemicals used in this study were of analytical grade. The main chemicals used in this study were: urea rectified (99.5%, India), ethanol (99.4%, Ethiopia), sodium hydroxide (ALPHA CHEMIKA, India), glacial acetic acid (99.5%), sodium chloride (NaClO_2), sulfuric acid (98%, India), potassium hydroxide (New Delhi, India), hydroperoxide (30%, Addis Ababa), sodium silicate (Bureau of India), and Epsom salt. All chemicals (Rankem, India) were purchased from Charcos, Addis Ababa, Ethiopia. Some of the common types of apparatus and instruments used in these experiments were: as follows: digital Soxhlet extractor, oven, analytical balance, disintegrator, reflux condenser, crushing machine, 500-gram high-speed electric powder grinder machine, thermometer, filtering crucible, crucible, magnetic stirrer, sieves, i50 FTIR spectrometer, TGA 55- TA Instruments, SEM (high-vac. SED PC-std., 15 kV), and XRD (SHIMADZU XRD-600).

2.2. The Study Area. Oxytenanthera abyssinica was collected from the west part of Ethiopia, Benishangul-Gumuz regional state, Metekel zone, Pawe woreda (Figure 1). It is located at an altitude of 1120 meters above sea level, between 36°20’ and 36°32’ longitude and 11°12’ and 11°21’ latitude. The climate of the woreda is humid and hot, with unimodal rainfall starting from May to October. The average annual rainfall in the area is 1586.32 mm, with temperatures between 19.4°C and 37.6°C [29]. From the overall land area of the woreda, the forest covers 30.6% of a hectare; among these, Oxytenanthera abyssinica is one of the plant species found in the forest [30].

2.3. Sample Collection and Preparation. Three-year-old stems of Oxytenanthera abyssinica were collected from the forest near Gilgel Beles town, Village 02. The plant stem was cut and repeatedly washed with water and then ground using a planner machine to produce chips. The chips were dried using sunlight for two weeks. Dried Oxytenanthera abyssinica chips were ground using an electric grain grinder mill and allowed to pass through a 40-mesh sieve and were retained by a 60-mesh sieve. The collected powdered material was stored in a plastic bag by assigning its name as ROA. This ROA sample is used for further experiments.

2.4. Isolation of Cellulose and Alpha-Cellulose. Ten grams of the ROA sample was used for 6 hours in Soxhlet extraction with a 2:1 benzene/ethanol ratio to remove waxes, resins, and other extractives. This extractive-free sample was assigned as DOA. The DOA was alkali-treated with 4, 5,
and 6% NaOH at room temperature (23°C), 75°C, 80°C, and 85°C for 3 hours with a fiber-to-solvent ratio of 1:10 with continuous stirring with a magnetic stirrer at 500 rpm to remove pectin, hemicellulose, and lignin. The fiber was filtered out of the NaOH solution and washed three times with distilled water until pH was 7 to produce AOA. For comparison, the alkaline treatment at room temperature for 24 hours also undergoes manual shaking every hour. A bleaching process was carried out by combining alkali peroxide bleaching stages (hydroperoxide, sodium hydroxide, sodium silicate, and Epsom salt) with sodium chlorite aqueous solution in buffer solution [31, 32]. The chemicals were mixed in the following order in a beaker: distilled water, 3% sodium silicate, 3% sodium hydroxide, 0.5% Epsom salt, and 4% hydrogen peroxide. The bleach solutions were then added to AOA, which had been heated for 40 minutes to the reaction temperature of 80°C. Following filtration and drying, the mixture of 100 mL aqueous chlorite (1.7% w/v NaClO₂), acetate buffer (2.7 g NaOH and 7.5 mL glacial acetic acid in 100 mL distilled water), and distilled water was added to the dried powder sample and then heated at 80°C for 4 hours. The bleached cellulosic fibers were then filtered and rinsed with water; finally, the fibers were air-dried at room temperature and assigned as BOA.

A method developed by Burton and Rasch [33], with some modification, was used to determine the alpha-cellulose content of Oxytenanthera abyssinica. 5-gram ROA was added to a 250 mL beaker; it was then immersed in a water bath at a constant temperature of 20°C. Once the sample had reached bath temperature, 17.4% NaOH solution was pipetted into the beaker and macerated with a stirring glass rod. After 30 minutes, the sample was filtered using a Buchner funnel. The filter paper residue was dried in the oven at 105°C until no additional weight change was observed. After this, the sample was cooled and 400 mL of distilled water was added. The mixture was filtered three times to make sure that the filter paper collected the entire residue. The residues were then soaked for 10 minutes in 100 mL of 20% acetic acid, the acetic acid was suctioned out, and 1 L of boiling water was added slowly in small amounts. The sample was maintained in a 105°C oven to eliminate the moisture. After drying, the difference in mass between total mass and filter paper mass is expressed as a percentage of alpha-cellulose in the sample.

2.5 Chemical Composition Analysis. Gravimetric analysis was applied to study the chemical composition of ROA, DOA, AOA, and BOA samples, such as cellulose, hemicellulose, lignin, holocellulose, and ash, based on the procedures used by Abe et al. and Then et al. [34, 35]. Figure 2 shows the procedure of chemical composition analysis. Acidified aqueous sodium chlorite (NaClO₂) was used to determine the amount of holocellulose. A sulfuric acid (H₄SO₄) solution was used to acidify 5% NaClO₂ solutions until the pH reached 4, with a 1:20 weight ratio of fiber-to-NaClO₂ solution. The sample residue was filtered out of the solution, rinsed with distilled water, and dried at 60°C. For the determination of the cellulose composite, a sample was soaked in 6% by weight potassium hydroxide solution at room temperature for 24 hours. The sample was then filtered, cleaned with distilled water, and dried at 60°C till it attained a constant weight. Hemicellulose is considered the difference between the values of holocellulose and cellulose. The lignin content of the sample was evaluated based on the TAPPI standard (T222) method. The sample was immersed in a solution of H₂SO₄ (72 wt%) for 2 hours at 30°C. The mixture was then diluted to 3% H₂SO₄ and refluxed for two hours. The solid residue was filtered, rinsed with distilled water, and dried in a 60°C oven until it attained a consistent weight.

2.6 Characterization

2.6.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis. After using 16 scans, FTIR was used to see the effect of the treatment based on the functional group change between samples such as ROA, DOA, AOA, and BOA. The IS50 FTIR spectrometer was used for the FTIR analysis. The spectra were taken in 4000–400 cm⁻¹ wave numbers.

2.6.2 X-Ray Diffraction (XRD) Analysis. An X-ray diffractometer (SHIMADZU XRD-6000) was used to determine the crystallinity degree, which utilized Cu K radiation at a current of 30 mA and a voltage of 30 kV, with a speed of 2°C/min and a scanning range of 10°-60°. The crystallinity degree of the samples was calculated using

\[
\text{CrI} (%) = \frac{I_{200} - I_{am}}{I_{200}} \times 100, \tag{1}
\]

where \(I_{am}\) denotes the amorphous peak or the intensity of baseline scattering, evaluated at 18.34°, and \(I_{200}\) denotes the crystallite peak, determined at 22.42° [36].

2.6.3 Thermal Gravimetric Analysis (TGA). A PerkinElmer Diamond TG/DTA thermal analyzer was used to test the material’s thermal stability. Experiments were conducted with a sample mass of roughly 10 mg, utilizing a linear
heating rate of 10°C/min and a constant nitrogen flow rate of 100 cm³/min in a temperature range of 25 to 785°C.

2.6.4. Scanning Electron Microscopy (SEM) Analysis. Scanning electron microscopy (high-vac. SED PC-std., 15 kV) was used to obtain SEM images of extracted cellulose. ImageJ software analysis was used to examine the image size of the samples.

2.6.5. Differential Scanning Calorimetry (DSC). DSC was used to assess the influence of pretreatment on degradation (DSC; TA Instruments, USA). The measurements were made with an alumina crucible with a pinhole and a nitrogen flow rate of 100 mL/min from ambient to 600°C at a constant 10°C/min heating rate.

2.7. Statistical Analysis. Results were subject to analysis of variance (ANOVA) using Origin 8 software. All measurements were carried out in triplicate (n = 3), and the values expressed are the mean of three repetitions ± standard deviations (SD). Significance was defined as a confidence limit of P ≤ 0.05.

3. Result and Discussion

3.1. Chemical Composition. The stem part of Oxytenanthera abyssinica was used as the raw material in this study. To obtain cellulose, this material was subjected to a multistep pretreatment process using Technical Association of the Pulp and Paper Industry (TAPPI) protocols. The chemical composition of the raw Oxytenanthera abyssinica fiber is shown in Table 1. The holocellulose content of raw Oxytenanthera abyssinica employed in this investigation was found to be equivalent to those reported by Razak et al. [37]. However, its amount is higher than the results reported by Liese and Tang [38], Correia [39], Razak et al. [37], and Cao et al. [40] and lower than the result reported by Sulaiman et al. [41] and Razak et al. [37]. The cellulose content (49.26 wt%) of Oxytenanthera abyssinica in this research is higher than that of Dendrocalamus brandisii (47.24 wt%) [42], Bambusa blumeana (40.3–45.1 wt%) [38], Bambusa tuloides (35.2 wt%) [39], Gigantochloa levis (33.8 wt%) [37], Passiflora edulis (44.64 wt%) [43], and Daphniphyllum oldhamii (47.1 wt%) [40]. The hemicellulose content of Oxytenanthera abyssinica is higher than that of Dendrocalamus brandisii [42], Bambusa vulgaris [41], Phyllostachys edulis [43], and Daphniphyllum oldhamii [40]. However, the hemicellulose of Bambusa tuloides is higher than that of Oxytenanthera abyssinica [39]. The large difference in cellulose composition might be because of the different soil types, age and maturity levels, and raw material sources. Genetics, age, location, growing conditions, anatomic structure, and plant maturity levels all influence the chemical composition of lignocellulosic materials.

Biomass must be treated to remove waxes, resins, and other impurities, reduce the refractory nature of lignocellulosic components, and separate cellulose effectively [45]. The pretreatment improves the biomass’s cellulose mass fraction and accessibility during the hydrolysis treatment stage by solubilizing hemicellulose and lignin. [46]. In this study, pretreatment procedures have been shown to change the chemical composition of ROA. 5.52 wt% of ROA extractives were removed using a 2 : 1 ratio of toluene and ethanol as a mixing solvent. The content of cellulose, hemicellulose, lignin, and ash was 49.26 ± 0.13 wt%, 21.31 ± 0.15 wt%,
20.63 ± 0.12 wt%, and 3.30 ± 0.11 wt%, respectively, in the ROA sample (Figure 3). In comparison to ROA, in DOA samples, cellulose and holocellulose increased to 54.11 ± 0.13 wt% and 74.83 ± 0.34 wt%, whereas the lignin, hemicellulose, and ash content decreased to 18.42 ± 0.02, 20.72 ± 0.21, and 2.74 ± 0.43 wt%, respectively. When compared to ROA and DOA, AOA has a higher percentage of holocellulose (89.07 ± 0.08 wt%), hemicellulose (12.1 ± 0.13 wt%), and cellulose (76.97 ± 0.03 wt%) and a lower percentage of lignin (7.28 ± 0.34 wt%) and ash (0.99 ± 0.09 wt%). This indicates the removal of lignin and hemicellulose from the sample via alkali treatment. Figure 3 shows that the BOA sample contains less noncellulose content (hemicellulose, lignin, and ash) than AOA, DOA, and ROA. This showed that the pretreatment removes more noncellulose content than others. The effect also resulted in a higher cellulose content (86.01 ± 0.02 wt%) in the BOA sample than in the AOA (76.97 ± 0.03 wt%), DOA (54.11 ± 0.13 wt%), and ROA (49.26 ± 0.13 wt%). The sample had zero ash content and 48.8 ± 0.23 alpha-cellulose content (Table 1). Figure 3 also demonstrates that AOA pretreatment resulted in the lowest yield (62.22 ± 0.53 wt%) when compared to DOA (87.11 ± 0.11 wt%) and BOA (80.41 ± 0.33 wt%) pretreatments. This demonstrated that alkaline treatment (6% w/v NaOH at 80°C for three hours) removes more noncellulose material than Soxhlet extraction (2:1 benzene/ethanol ratio, 6h) and bleaching (alkali peroxide stages followed by aqueous chlorine in buffer solution). In general, these findings clearly illustrate that by removing noncellulose content in each step, pretreatment can increase the content of cellulose in the order of ROA (49.26 ± 0.13), DOA (54.11 ± 0.13), AOA (76.97 ± 0.03), and BOA (86.01 ± 0.02) (Figure 3).

3.2. Alkaline Treatment. Similar to other lignocellulosic reinforcing plants, bamboo is a multicellular composite fiber. Their fibers are made up of cellulose microfibrils of various fibrillar orientations that are bonded together and surrounded by lignin and hemicellulose [47]. The overlapping nature of the fibers gives a mesh-like structure. The NaOH reacts with the hydroxyl groups of these cementing substances, usually hemicellulose. The reaction destroys the cellular structure and causes the fiber’s hydrophilic characteristic to deteriorate and decrease the content of hemicellulose [48]. The following reaction will occur on the surface of the bamboo fiber to remove noncellulosic materials using alkaline treatment:

\[
\text{Bamboo-OH} + \text{NaOH} \rightarrow \text{Bamboo-O}^- \text{Na}^+ + \text{H}_2\text{O}.
\]

Figure 4 shows how Soxhlet extraction and alkaline treatment affect fiber morphology using an optical microscope. As observed in the image, a more cementing material (lignin, hemicellulose, wax, and others) is present in high quantities in sample A. In sample B, some fibers are observed free from this cementing substance. Less noncellulose material was observed in the sample compared to sample A. Since sample C was obtained by alkaline treatment, there was less cementing material than in samples A and B. These showed that chemical treatment could change the morphology of the *Oxytenanthera abyssinica* fiber.

The main purpose of the pretreatment process is to increase cellulose crystallization, remove or change the content of lignin and hemicellulose, break down complex carbohydrate polymers, and increase its surface area [49]. The alkali pretreatment method is still popular because of its advantages, such as low chemical cost and high lignin removal [50]. Another advantage of the alkaline treatment of natural fibers is that it removes wax, pectin, lignin, and certain hemicellulose from the surface [51]. It may cause the smooth surface of natural fibers to become rough, resulting in improved mechanical bonding [52]. In most studies, sodium hydroxide (NaOH) is used as an alkaline pretreatment [53]. Table 2 shows the relationships between NaOH concentration, reaction time, and temperature with lignin,

### Table 1: Chemical composition comparison of ROA to other bamboo species.

| Samples                        | Cellulose (wt%) | Hemicellulose (wt%) | Lignin (wt%) | Holocellulose (wt%) | Alpha-cellulose (wt%) | Ash (wt%) | Reference |
|--------------------------------|-----------------|---------------------|--------------|---------------------|-----------------------|-----------|-----------|
| Oxytenanthera abyssinica       | 49.26 ± 0.13    | 21.31 ± 0.15        | 20.63 ± 0.12 | 70.57 ± 0.28        | 48.8 ± 0.23           | 3.30 ± 0.11| This study|
| Gigantochloa apus              | RNF*            | RNF*                | 22.41        | 63.23               | 47.56                 | 6.09      | [44]      |
| Dendrocalamus asper            | RNF*            | RNF*                | 25.27        | 68.25               | 48.60                 | 2.13      | [44]      |
| Gigantochloa robusta           | RNF*            | RNF*                | 23.86        | 56.81               | 44.36                 | 1.66      | [44]      |
| Dendrocalamus giganteus        | RNF*            | RNF*                | 23.85        | 65.96               | 46.88                 | 3.70      | [44]      |
| Dendrocalamus brandisii        | 47.24           | 23.85               | 23.84        | RNF                 | RNF*                  | 1.37      | [42]      |
| Bambusa blumeana              | 40.3            | RNF*                | 20.5         | 65.7                | RNF*                  | RNF*      | [38]      |
| Bambusa tulidoides            | 35.2            | 32.0                | 25.5         | 67.2                | RNF*                  | 3.7       | [39]      |
| Gigantochloa brang            | 51.58           | RNF*                | 24.83        | 79.94               | 51.18                 | 1.25      | [37]      |
| Gigantochloa levis            | 33.8            | RNF*                | 26.50        | 85.08               | 33.81                 | 1.30      | [37]      |
| Goniothalamus scortechinii     | 46.87           | RNF*                | 32.55        | 74.62               | 46.87                 | 2.84      | [37]      |
| Bambusa vulgaris              | 51.25           | 29.32               | 22.29        | 85.50               | RNF*                  | RNF*      | [41]      |

RNF* in the table means "result not found."
Figure 3: Chemical composition of Oxytenanthera abyssinica at different pretreatments. C: cellulose; He: hemicellulose; L: lignin; Ho: holocellulose; A: ash; Y: yield.

Table 2: The evaluation of the relationships between NaOH concentration, reaction time, and temperature with lignin, hemicellulose, and cellulose content after alkaline treatment.

| Sample code | [NaOH] (wt%) | Temperature (°C) | Reaction time | Chemical compositions (wt%) |
|-------------|--------------|-----------------|---------------|-----------------------------|
|             |              |                 |               | Cellulose                  | Hemicellulose               | Lignin                       |
| 6R24        | 6            | 23              | 24 hr * 1     | 67.47 ± 0.15               | 15.8 ± 0.20**               | 13.78 ± 0.09                 |
| 5R24        | 5            | 23              | 24 hr * 1     | 63.94 ± 0.31               | 17.01 ± 0.25*               | 15.18 ± 0.10                 |
| 4R24        | 4            | 23              | 24 hr * 1     | 58.61 ± 0.16               | 20.28 ± 0.15               | 17.33 ± 0.12                 |
| 6SF3        | 6            | 75              | 3 hr * 3      | 69.80 ± 0.08*              | 17.19 ± 0.23*              | 10.33 ± 0.12                 |
| 6EF3        | 6            | 85              | 3 hr * 3      | 72.69 ± 0.08               | 16.27 ± 0.18**             | 7.36 ± 0.06                  |
| 4EN3        | 4            | 80              | 3 hr * 3      | 69.74 ± 0.02*              | 18.07 ± 0.22               | 9.46 ± 0.12                  |
| 5EN3        | 5            | 80              | 3 hr * 3      | 74.76 ± 0.02**             | 13.43 ± 0.02               | 8.56 ± 0.13                  |
| 6EN2        | 6            | 80              | 2 hr * 3      | 73.99 ± 0.02               | 14.39 ± 0.02               | 8.38 ± 0.08                  |
| 6EN3        | 6            | 80              | 3 hr * 3      | 76.97 ± 0.03               | 12.1 ± 0.13                | 7.28 ± 0.34                  |
| 6EN4        | 6            | 80              | 4 hr * 3      | 74.99 ± 1.3**              | 14.06 ± 0.25***            | 7.14 ± 0.01                  |

All data were determined as triplicate, and values in the same column with the identical superscript (*, **, or *** ) are not significantly different (P ≥ 0.05), and values in a column without a superscript are significantly different from each other (P ≤ 0.05).
ponents have been removed from OA fib noncellulosic components (lignin and hemicellulose) have The white color of the bleached sample showed that the various treatments of the isolated cellulose contains more –OH groups [59]. The peak at 1726 cm−1 present in ROA and DOA is due to the out-of-plane C-O stretch of the lignin aryl group [62]. Due to the removal of lignin and hemicellulose during the treatments, the peak at 1249 cm−1 had been removed in the spectra of the AOA and BOA. This finding is supported by the work of Rayung et al. [63] and Razak et al. [64]. The free O-H stretching vibration of the OH group was determined by the wideband range of all samples such as ROA, DOA, AOA, and BOA at 3332 cm−1 (Figure 7) [65]. At 2906 cm−1, C-H group stretching was represented by the band at 2906 cm−1 [58]. The band at about 3332 cm−1 is narrower and has a higher intensity than the untreated fiber (ROA), confirming that the isolated cellulose contains more –OH groups [59]. The peak at 1726 cm−1 of ROA is because of the C=O stretch of the uronic and acetyl ester groups of pectin and hemicellulose or the ester leakage of the carboxyl groups (fumaric and ferulic acid) of hemicellulose and/or lignin [60, 61]. However, this peak disappears in the alkaline-treated fiber (AOA) and bleached fiber (BOA) due to lignin and/or hemicellulose removal. The absorption peak of 1249 cm−1 present in ROA and DOA is due to the skeletal vibration of the C-O pyranose ring. An increase in the intensity of the peak in samples

hemicellulose, and cellulose content after alkali treatment. The increase in NaOH concentration (4 to 6% w/v) enhanced the percentage composition of cellulose by 7.23 wt% and decreased the content of hemicellulose by 5.97 wt%, keeping temperature and reaction time constant at 80°C and 3 hours. Raising the reaction temperature from 75 to 80°C while keeping the NaOH concentration (6% w/v) and reaction time (3 h) constant increases the percentage of cellulose composition by 7.17 wt% and decreases hemicellulose composition by 5.09%. Increasing the alkali solution concentration from 4 to 6% w/v while maintaining the reaction time (3 h) and temperature (80°C) constant reduced the content of hemicellulose and lignin significantly by ~6 wt% and 2.18 wt%, respectively (Table 2). Changing the reaction time from 2 to 4 hours while maintaining the same NaOH concentration (6% w/v) and temperature (80°C) resulted in a nonsignificant change (P ≥ 0.05) in cellulose concentration from 73.99 ± 0.02 to 74.99 ± 1.29. The optimal condition for yielding the highest amount of cellulose is to treat the Oxytethanta abyssinica fiber with a 6% w/v concentration of NaOH at 80°C for 3 hours. Alkaline treatment at room temperature (23°C) for 24 hours resulted in lower cellulose and hemicellulose concentration and higher lignin content than other temperature pretreatment settings (75, 80, and 85°C). In general, the data in Table 2 show that the sample at low temperature (23°C) and short reaction time (2 hours) had the highest lignin (12.3 ± 0.12 wt%) and hemicellulose content (20.45 ± 0.17 wt%) and the lowest cellulose content (55.44 ± 0.25 wt%) than other samples (6R24, 5R24, 6SF3, 6EF3, 4EN3, 5EN3, 6EN2, 6EN3, and 6EN4).

3.3. Characterization

3.3.1. Morphological and Chemical Analyses. Figure 5 shows pictures of four samples, such as ROA, DOA, AOA, and BOA, produced after treatment phases such as powdering of Oxytethanta abyssinica, dewaxing of ROA, alkaline treatment of DOA, and bleaching of AOA. The color of the fiber varies from light brown to yellow (AOA), then to white after bleaching (BOA). The color of ROA after Soxhlet extraction is changed to light brown in the DOA sample. The white color of the bleached sample showed that the noncellulosic components (lignin and hemicellulose) have been eliminated from the fiber [54]. Thus, the color changes due to chemical treatment, indicating that some fiber components have been removed from OA fibers.

SEM was used to examine the external morphology of the various treatments of Oxytethanta abyssinica. Different chemical treatments performed on the fibers affect fiber morphology by exposing the fiber surface to removable noncellulosic materials such as wax, pectin, lignin, hemicellulose, and impurities from the fiber [55]. Figure 6 shows SEM images of chemically treated ROA, AOA, and BOA fibers. The image revealed that chemical treatment changed the morphology of the treated material. This change in the sample morphology is the main indication of the effectiveness of chemical treatment [56].

The ROA sample surface is covered with impurities, waxy components, and a cement material such as hemicellu-
showed an increase in crystallinity [70]. Thus, BOA showed more crystallinity than AOA, DOA, and ROA. The most important absorption band is obtained at 892 cm\(^{-1}\) due to the presence of the \(\beta\)-glycosidic linkage between the anhydroglucose units in cellulose. The band intensity increased in the order of ROA, DOA, AOA, and BOA because the percentage of a cellulosic constituent has increased. This implies that the samples contain more pure cellulose [71].

3.3.3. X-Ray Diffraction (XRD) Analysis. The X-ray spectra of the samples (ROA, AOA, and BOA) after different treatment stages are shown in Figure 8. The XRD image of the three samples showed three different peaks at 15.96°, 22.42°, and 34.6°, which are indicative of natural crystalline structures such as cellulose I. The diffraction of planes (110), (200), and (004) is responsible for these peaks in their respective manner. The polymorphs of *Oxytenanthera abyssinica*, as well as the isolated cellulose fibers, were confirmed via crystallographic analysis using XRD. French [72] got a comparable result from the bamboo pulp fibers. He obtained two weaker peaks (040) and (110) centered at 34.1° and 16.2°, respectively, and a powerful sharp peak (002) at 22.6°. In the current study, the XRD peak did not show differences in peak positions or lattice characteristics between the three samples (ROA, AOA, and BOA). These revealed that neither swelling nor polymorph change occurred during the chemical purification procedure. However, there is a higher intensity variation at 22.42° due to the diffraction of
planes at (200). An increase in sample crystallinity is the primary cause of this intensity variation [73]. Crystallite levels increase with the amount of cellulosic material in the sample [73]. The crystalline rearrangement into a more ordered structure is another possibility for the change in peak intensity [56].

Table 3 shows the crystallinity index (CrI) of ROA, AOA, and BOA at various stages of treatment. The results

| Samples | Peak position 2θ (°) (amorphous) | Peak (002) position 2θ (°) | CrI (%) |
|---------|----------------------------------|-----------------------------|--------|
|         | Degree                           | Intensity (I_{am})          | Degree | Intensity (I_{002}) |        |
| ROA     | 18.32                            | 693                         | 22.02  | 1292               | 46.36  |
| AOA     | 18.1                             | 574                         | 22.2   | 1287               | 55.40  |
| BOA     | 18.16                            | 463                         | 22.2   | 1289               | 59.89  |

Figure 7: FTIR spectra of ROA, DOA, AOA, and BOA.

Figure 8: XRD pattern of ROA, AOA, and DOA.
showed that ROA, AOA, and BOA have 46.36, 55.43, and 59.89% CrI values, respectively. When comparing the CrI value of cellulose isolated from *Oxytenanthera abyssinica* (59.89%) with other bamboo plants such as *Phyllostachys heterocycla* (57.8%), it has a higher value [74]. However, it has lower CrI values than cellulose derived from rice straw (71%) and potato tuber (68%) [75]. Due to high amorphous content, the ROA sample exhibited a lower CrI value. After alkaline treatment and bleaching, the value increased by 9.07% and 13.53%, respectively. The increase in the CrI value implies that impurities such as wax, hemicellulose, and lignin were eliminated from ROA [35, 76].

3.3.4. Thermogravimetric Analysis (TGA). Thermogravimetric analysis is performed in this study to investigate the samples’ thermal behavior due to chemical treatment. Thermal behavior is determined by the samples’ chemical composition, structure, and crystallinity [77]. The relative amounts of amorphous and crystalline cellulose in the samples affect the crystallinity of the samples. As crystallinity increases...
due to chemical treatment, so does the degradation temperature [55]. Since lignin, hemicellulose, and cellulose have different chemical structures, they are predicted to decompose at different temperatures [55]. As a result, different thermogravimetric curves are produced during the chemical treatment of OA [78].

Figure 9 shows the TGA and DTG curves of ROA, AOA, and BOA samples. Due to the decomposition of the lignocellulosic components, the main weight loss was recorded at around 250 to 380°C as shown in TGA and DTG peaks [79]. Studies showed that cellulose decomposition temperature mainly occurs around 315°C to 400°C, hemicellulose decomposition begins at 220°C and continues up to 315°C, and lignin decomposition takes place between 200°C and 700°C [80]. Thus, the decomposition temperature that ranged from 210°C to 400°C in Figure 9 was due to cellulose, hemicellulose, and lignin decomposition. The decomposition temperature greater than 400°C is mainly due to lignin decomposition; ROA, AOA, and BOA showed decomposition temperatures around 220, 280, and 290°C. This is an
indication mainly of the decomposition of hemicellulose [80]. Since ROA has more significant hemicellulose content (Figure 3) than AOA and BOA, it exhibited lower decomposition temperature (220°C) because hemicellulose degrades quickly at low temperatures compared to cellulose. The presence of acetyl groups in hemicellulose may be responsible for its lower thermal stability [80]. At higher temperatures than hemicellulose decomposition, cellulose decomposition was observed at around 335°C for ROA, AOA, and BOA as we observed in the DTG curve (Figure 9(b)). BOA and AOA have a higher amount of decomposition temperature than ROA; this is due to their containing a higher percentage of cellulose (Figure 3). Since cellulose comprises a long linear chain of thermally stable polysaccharides, it requires a higher temperature for decomposition [81]. The temperatures above 400°C shown in both the DTG and TGA curves (Figures 9(a) and 9(b)) indicate the decomposition of lignin and other impurities. Since the three samples have different chemical compositions, there was a difference in the amounts of char residuals [82]. The DTG curve also showed the decomposition of ROA, BOA, and AOA at 333, 335, and 340°C, respectively. The char residuals of BOA, ROA, and AOA samples were 570, 590, and 600°C, respectively. This study showed that the thermal decomposition in TGA is due to hemicellulose, cellulose, and lignin decomposition. The above data demonstrated that chemical treatment alters the chemical composition of *Oxytenanthera abyssinica*, resulting in thermally stable BOA samples.

3.3.5. Differential Thermal Analysis. Figure 10 shows the differential thermal analysis (DTA) spectra of BOA, AOA, and ROA samples. The sample’s moisture loss is correlated with the peak of around 80°C. Around 216°C and 350°C, hemicellulose and cellulose decomposition occurred, respectively. The removal of lignin was observed in DTA spectra from 200 to 800°C. A related study showed that the decomposition of hemicellulose occurred at a temperature range of 220 to 315°C, cellulose of 300 to 400°C, and lignin over a wider temperature range of 150 to 900°C [80]. As seen in Figure 10, there is an intense peak at 221°C; this confirmed that ROA contains hemicellulose and/or lignin content more than AOA and BOA. The other peak at 355°C showed that BOA degrades at a higher temperature compared to AOA (354°C) and ROA (345°C). This demonstrated that the untreated sample (ROA) contains the least cellulose content, whereas the alkaline-treated (AOA) and bleached samples (BOA) possess more cellulose content. The highest decomposition of BOA comes from increasing the degree of crystallinity [83]. Since BOA has the highest CRI value (59.89%) (Figure 8) than AOA (55.40%) and ROA (46.36%) samples, it gives a higher decomposition temperature (355°C); in contrast, ROA has the least decomposition temperature (336°C).

3.3.6. Differential Scanning Calorimetry Analysis. Differential scanning calorimetry (DSC) analysis can be used to determine the chemical activity in the fiber as heat is applied [83]. In this study, the thermal behavior of cellulose, hemicellulose, and lignin was determined by differential scanning calorimetry (DSC) analysis on the ROA and BOA (Figure 11). In the DSC peak, both of the samples showed large endothermic peaks in the temperature range of 28 to 115°C, corresponding to the escape of water absorbed by the fiber. The absence of visible exothermic or endothermic peaks between 120 and 265°C for BOA and 130 and 256°C for ROA indicates that the fibers were thermally stable in this range. The endothermic peak of BOA (65.3°C) is greater than that of ROA (52.3°C). This demonstrates that BOA has higher lignocellulose content than ROA (Figure 11). According to the DSC result obtained by Aziz and Ansell [84], lignin degrades at around 200°C, but other polysaccharides such as hemicellulose and cellulose decomposed at greater temperatures. Based on this information, in Figure 11, peaks above 200°C suggest cellulose and hemicellulose breakdown. The thermal degradation of lignin causes the first exothermic hump in the DSC curve of ROA, around 174.6°C and 193.6°C. The other endothermic peak indicates the decomposition of hemicellulose at 275°C and 310°C for BOA and 230°C for ROA. The decomposition of cellulose was observed in the TGA peak at 324 and 350°C, respectively. The cellulose degradation temperature increased beyond 324°C for ROA and 350°C for BOA. This finding suggests that pretreatments break down amorphous polysaccharides initially, leaving crystalline and heat-resistant cellulose molecules behind.

4. Conclusion

Cellulose is the substance that makes up most of a plant’s cell walls. Two-step processes were used to isolate cellulose from *Oxytenanthera abyssinica* fibers. First, we have alkaline treatment with 6% NaOH and then two bleaching stages (alkali peroxide+aqueous chlorite in buffer solution). The highest amount of cellulose and holocellulose was exhibited in bleached samples with a yield of 86.01 ± 0.02 wt% and 97.61 ± 0.18 wt%, respectively. The lowest amount of cellulose was observed in ROA, with a yield of 49.26 ± 0.13 wt%. By removing noncellulose material, cellulose content increases in the order of ROA, DOA, AOA, and BOA. The data from FTIR measurements showed that the chemical treatments removed hemicellulose and lignin from the samples. The TGA and DTG curves showed that the main weight loss was found in the range of 280-360°C. XRD showed that more crystalline cellulose with a crystallinity index value of 59.98% was obtained using pretreatment procedures. These findings show that *Oxytenanthera abyssinica* is a viable renewable source for cellulose production. Even though *Oxytenanthera abyssinica* is widely grown in Ethiopia, there were no sufficient studies in this area and no works have been carried out for the isolation and characterization of cellulose from the plant. Thus, this study will encourage more research to use the plant as a good source of cellulose for different applications such as biofilters for polymer matrices, green biocomposite reinforcement, food packaging, and cellulose nanocrystal preparation.

Data Availability

Data are available on request.
Conflicts of Interest
The authors declare that they have no conflicts of interest.

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