

Research Article

Proximate Chemical Analysis and Effect of Age and Height of *Oxytenanthera abyssinica* on Fiber Morphology and Chemical Compositions for Pulp and Paper Production Potential

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Received 17 January 2023; Revised 5 March 2023; Accepted 8 March 2023; Published 20 March 2023

Academic Editor: Yiqi Yang

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This study examined the chemical composition, fiber morphology, and physical properties of *Oxytenanthera abyssinica* culm to assess its pulping potential. Technical Association of the Pulp and Paper Industry (TAPPI) and Franklin's methods have been used for experiments. The statistical analysis showed that the chemical composition of *O. abyssinica* is influenced by the age of the plant. The amount of cellulose in the culm increases with age, while hemicellulose and lignin contents decrease with age. The average chemical content of the three years aged *O. abyssinica* was 49.26 ± 0.13 wt%, 21.31 ± 0.15 wt%, and 20.63 ± 0.12 wt% for cellulose, hemicellulose, and lignin contents, respectively. A significant difference exists between 1, 2, and 3-year aged plants ($P < 0.05$) in cellulose, lignin, ash, and extractive content. The position of the culm also affects the fiber morphology of *O. abyssinica*. The fiber's length, diameter, cell wall thickness, and lumen diameter increase from top to bottom, whereas the flexibility and slenderness ratio decrease. The average fiber morphology of *O. abyssinica* was flexibility ratio (0.72 ± 0.10), Runkel ratio (0.35 ± 0.10), slenderness ratio (109.98 ± 0.21), lumen diameter (15.63 ± 0.03 μm), cell wall thickness (2.74 ± 0.03 μm), fiber length (2.40 ± 0.10 mm), and fiber diameter (21.83 ± 0.09 μm). The above data showed that the mean value of the fiber length of the plant is greater than 1.5 mm, the Runkel ratio was less than 1, and the slenderness ratio was greater than 70 standard values. The result also showed that the average bulk density and the moisture content were 660 kg/m^3 and 9.6%, respectively. Although *O. abyssinica* is widely grown in the study area, no comprehensive studies have been carried out on fiber morphology, chemical composition, and physical properties based on age and height. Thus, this research was carried out to study the plant's fiber characteristics to assess its suitability for pulp and paper production. Based on the above data, the 3-year aged bottom part of *O. abyssinica* is recommended for more yield pulp and high-quality paper production than the first and second-year aged plant.

1. Introduction

Bamboo is one of the non-wood forest resources with significant potential as a substitute for wood resources due to its high productivity, rapid growth, and ease of reproduction [1]. It grows rapidly in tropical and subtropical regions and is called “green

gold” because of its diversity [2]. Bamboo has received much ecological and economic attention in recent decades due to its rapid growth, compact inheritance, and superior mechanical strength compared to other plants [2, 3]. Around the world, more than 1,500 species of bamboo have been found in 90 genera, it covering 36 million hectares (ha) from latitudes 460°N to

470°S, 4000 m above sea level [4]. It occurs in many countries, such as South America (Colombia, Ecuador, and Peru), Central America (Honduras, Mexico, and Costa Rica), and Asia [5]. In Africa, Ethiopia has the largest bamboo forest [6].

Different bamboo species are very important alternatives for pulp and papermaking properties. The rising demand for wood and fiber and the decreasing availability of wood supplies have prompted researchers to look for non-wood plants with fiber properties similar to wood. As a result, non-woody plants like bamboo could serve as an alternate source of pulp and papermaking raw materials. According to various authors, pulp from non-wood sources has many benefits, including simple pulping capabilities, suitable fibers paper, and high-quality bleached pulp [7]. It can be utilized as a viable replacement for ever-diminishing forest timber resources. Non-woody plants give different advantages, such as short growth cycles, they are fast-growing, they need moderate irrigation and fertilization, and low lignin content resulting in the reduction of energy and chemicals used during pulping process [8].

Oxytenanthera abyssinica is one of the non-woody plants. It is a commercially and environmentally important bamboo species native to Ethiopia, accounting for around 85% of the country's total bamboo acreage [9, 10]. It covers around 850, 000 hectares in the country's center, southwest, and southern regions [10]. It is a fast-growing, high-yielding renewable resource. It is low-cost, quick-growing, widely available, and has physical and mechanical qualities similar to wood [11]. Even though the plant has good properties for pulp and paper raw materials, there need to be more studies carried out in the country. There is no study on *O. abyssinica* fiber morphological variation based on the plant's age and culm position in Ethiopia. Thus, this study evaluates the proximate chemical analysis, fiber properties of the plant based on age (1, 2, and 3 years) and location of the culm (top, middle, and bottom) for an alternative source for pulp and paper potential in the country. Studies showed that the most important aspects determining the characteristics of paper and pulp production were the type of bamboo species, age of the plant, origin, and culm height [12]. Because the most important chemical compositions that determine the pulping properties, such as cellulose, hemicelluloses, and lignin, highly depend on these characteristics.

2. Materials and Methods

2.1. Material. Analytical-grade substances were employed in this study. Ethanol (99.4%, Ethiopia), sodium hydroxide (ALPHA CHEMIKA, India), nitric acid (50%), safranin solution (Labchem), xylene, Canada balsam (ALPHA CHEMIKA, India), and sulfuric acid (98%, India) were the main chemicals used in this study. All chemicals (Rankem, India) were purchased from Charcos, Addis Ababa, Ethiopia. Analytical balance, reflux condenser, crushing machine, thermometer, filtering crucible, crucible, magnetic stirrer, different size sieves, Motic BA210 microscope, water bath, Whatman grade 1 filter paper, Leica sliding microtome, and 3D light microscope were some of the common types of instruments and apparatus used in these experiments.

2.2. The Study Area. Lowland Ethiopian bamboo (*O. abyssinica*), 1- to 3-year age stems, were taken from the west part of Ethiopia, Metekel zone in Pawe Woreda (Figure 1). It is located at an elevation of 1120 m above sea level, with longitudes of 36 20' to 36 32' and latitudes of 11°12' to 11°21'. It has a humid and hot climate, with temperatures ranging from 19.4°C to 37.6°C [13].

2.3. Sample Collection, Identification, and Use. According to TAPPI (Technical Association of Pulp and Paper Industry) (2002) Standard Method [14], 20 *O. abyssinica* plants with an average age of 3 years were randomly selected from a natural forest found near village 7 town, Pawe Woreda for fiber characterization test. The plant's culms (9 m) were cut above the second node, or around 30 cm, because cutting the culms below this height will slow the growth of the remaining culms. The culms were divided into three equal lengths, each measuring 3 m, for the bottom, middle, and top segments to examine the culms' fiber characteristics.

For proximate chemical analysis, age (1, 2, and 3 years), *O. abyssinica* were randomly identified and selected based on age. The culms of identified *O. abyssinica* were cut with axes; after removing the branches and top parts of the plant, the culms were rinsed repeatedly with water and then chopped into chips with a planer machine. The chips were dried in the sun for 2 weeks. The dried chips of *O. abyssinica* were crushed, and the mesh size of over 60 mesh and under 40 mesh size were collected and stored in polyethylene bottles for proximate chemical analysis.

The identification of the plant species was carried out by taxonomists at Addis Ababa University, College of Natural Sciences Department of Biology and Biodiversity Management, and the use of plant parts in the present study complies with international, national, and institutional guidelines [15]. Permission was received from the Pawe Woreda Agricultural and Rural Development Office to harvest plant material.

2.4. Physical Properties of *O. abyssinica*

2.4.1. Moisture Content. Sample blocks representing the 3 years *O. abyssinica*, at the top, middle, and bottom position with six replicates, were used to measure moisture content. All sample blocks were cut from fresh culms with a wall thickness of 10 × 10 mm × culms wall thickness. They were weighed and dried in a 105°C oven for 48 hours until a consistent weight was achieved. Afterward, the sample blocks were placed in a desiccator for 30 minutes to cool down before being re-weighed. The samples were weighed using a digital scale with a 0.001 g precision and oven-dried at 105°C for 72 hours to achieve a constant weight value. The moisture content was determined using ASTM 5229 using equation (1) [16]

$$\text{Moisture content} = \frac{\text{GM}_{\text{oven}} - \text{ODW}}{\text{ODW}} \times 100\%, \quad (1)$$

where: GW = green weight (the weight of sample before drying); ODW = the weight of a sample after drying with oven (oven-dry weight).

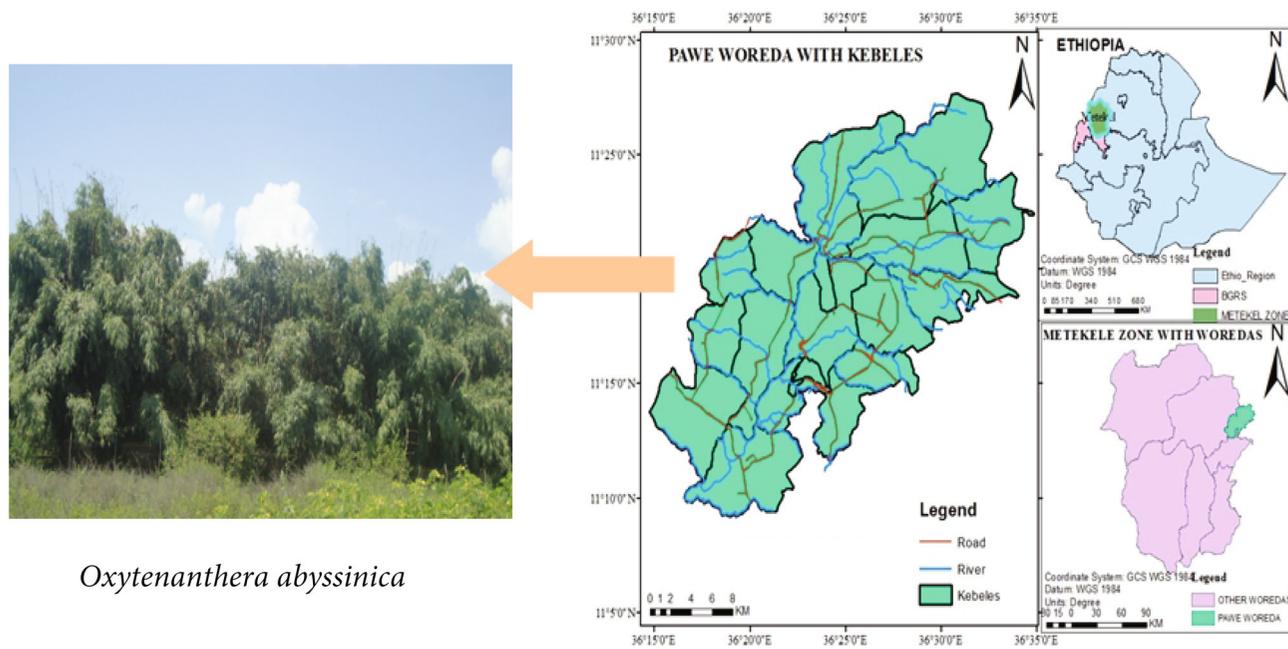


FIGURE 1: A region in Ethiopia where *Oxytenanthera abyssinica* was collected.

2.4.2. Basic Density. Sample blocks representing the 3 years *O. abyssinica*, at the top, middle, and bottom position (3 cm each) with six replicates, were used to measure moisture content (Figure 2). After removing the outer part of the plant samples, the middle region of each internode was taken from the top, middle, and bottom culms segments for basic density tests. Six sample block was cut to a culms wall thickness of 30 cm × 30 cm for each position. The sample blocks (18 samples) were oven-dried for 48 hours at 103°C until they attained a predetermined weight. The dry weight of the oven was calculated by measuring the weight of the sample block. The oven-dry weight and green volume were used to calculate density. The water displacement method was used to determine the green volume of the sample. Equation (2) was used to compute basic density [16]

$$\text{Basic density} = \frac{M_o}{V_2}, \quad (2)$$

where M_o is the oven-dried mass of each sample in g, and V_2 is the fully saturated (maximum) volume in ml.

2.5. Fiber Morphology of *O. abyssinica*. TAPPT standards performed proximate chemical analysis were used: Technical Association of the Pulp and Paper Industry (TAPPI) T203 om 99 for alpha-cellulose content, TAPPI T211 om-93 for ash content, TAPPI T207 cm-99 for water solubility, TAPPI T222 om-02 for lignin content, TAPPI T212 om-98 for 1% NaOH solubility, and TAPPT T204 cm-97 for alcohol-benzene solubility. The wise technique was used to analyze holocellulose. The cell lumen, cell wall thickness, fiber breadth, and fiber length were all measured after fiber maceration using Franklin's method. Three derived values were also computed based on the fiber dimensions such as slenderness ratio (fiber length/fiber diameter), flexibility ratio [(fiber lumen diameter/

fiber diameter) × 100], and Runkel ratio [(2 × fiber cell wall thickness)/fiber lumen diameter] [17, 18]. The findings were then examined to see if *O. abyssinica* might be used to make pulp and paper.

2.6. Fiber Dimensions Measurement Procedure. The width and length of the fibers were examined using the following methods (Figure 3). The maceration process was performed using a match-size sample and nitric acid (50%). For fiber separation, matchstick-sized samples were placed in test tubes, immersed in nitric acid solution, and kept at 70°C for 5–6 hours. After cooling, the nitric acid was drained, and the fibers were rinsed in distilled water before being separated on the Whatman grade 1 filter paper [19]. A Motic BA210 microscope with a camera was used to take the images.

2.7. Cell Dimension Measurement. Slices with a thickness of 20 μm were cut with a Leica sliding microtome for cell measurement [20]. To eliminate excess safranin solution, the slices were soaked for 1 minute in a safranin solution (1 g/100 ml water) and alcohol concentrations of 25%, 50%, and 75%, respectively. Slices were submerged in xylene for 1 minute before being placed on a standard 7.5 cm × 2.5 cm slide. Finally, a small amount of Canada balsam was dropped and left to dry before applying the slide cover. The lumen diameters and cell wall thickness were measured using Motic software, and an image was recorded with a camera attached to a Motic BA210 microscope (Figure 3).

2.8. 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 size image of the samples.

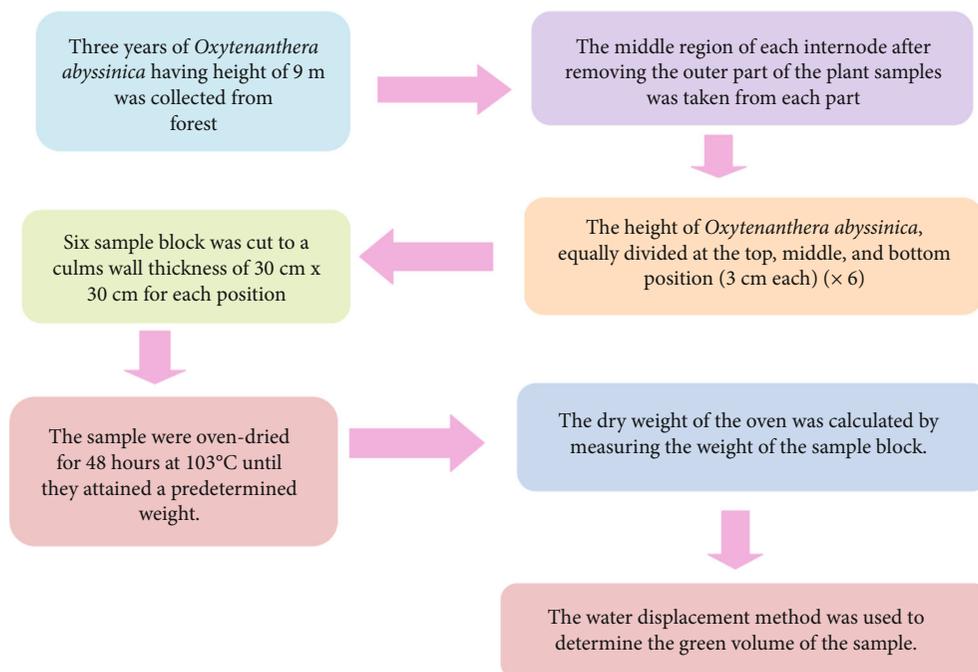


FIGURE 2: Procedures used to measure the basic density of *Oxytenanthera abyssinica*.

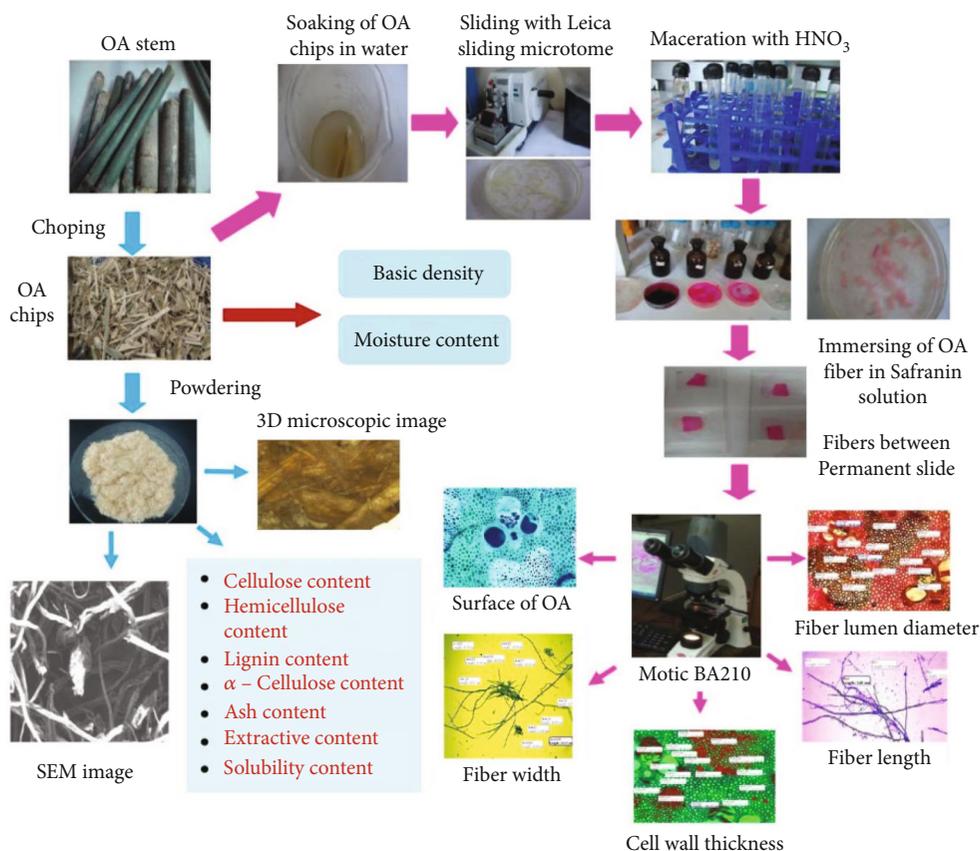


FIGURE 3: *Oxytenanthera abyssinica* (OA) fiber morphological characterization procedures.

2.9. *Statically Analysis.* Analysis of variance (one-way ANOVA), p -value, confidence limit (CI), and standard deviation (SD) was performed using Origin 8 and Microsoft

excels software. Measurements for cellulose, lignin, hemicelluloses, ash, extractives, solubility in alcohol, benzene, 1% NaOH, hot water, and cold water were performed three

times ($n = 3$) for 1-, 2-, and 3-year age plants. For each sample, more than 14 readings were taken from the plant's bottom, middle, and top positions to measure the fiber morphology (Tables 1 and 2). Six repetitions of each experiment were utilized to measure physical properties such as basic density and moisture content. The average results with SD are reported in the manuscript. A 95% confidence limit of P value less than or equal to 0.05 was used to determine significance. Mean comparisons were performed using Tukey's test.

3. Result and Discussion

3.1. Physical Properties of *O. abyssinica*

3.1.1. Moisture Content. The species type, the age of the culm, the place of growth, the length of the culm, and the thickness of the culm wall all affect how much moisture is present in a particular bamboo species [21]. This study measured the moisture contents at the top, middle, and bottom of a 3-year-old *O. abyssinica* bamboo species. The result showed that 9.37 ± 0.240 wt% mean moisture content was observed. The proportion of moisture content decreased from the bottom to the top as the culm height of *O. abyssinica* increased. The top, medium, and bottom parts of *O. abyssinica* moisture content were 8.73 ± 0.21 wt%, 9.4 ± 0.32 wt%, and 9.99 ± 0.21 wt%, respectively. There is no significant variation ($p < 0.05$) in moisture content between the plant's top, middle, and bottom parts. This showed that the position of the culm does not significantly affect the moisture content. The moisture content of the culm dropped as the height of the culm increased, which is consistent with previous findings from other bamboo species [22]. In the current study, the average moisture content is significantly lower than other bamboo species, such as *Dendrocalamus asper*, *Bambusa vulgaris*, *Gigantochloa scortechinii*, and *Schizostachyum grande*. For instance, the moisture content of the top section of the above bamboo species was found to be 20.83 wt%, 15.29 wt%, 16.09 wt%, and 23.36 wt%, respectively [23]. This shows that, in comparison to the other bamboo species described above, *O. abyssinica* has a higher potential for paper production. This is because moisture reduces the bonds between the fibers formed in the paper-making process and reduces paper strength [24]. According to studies, paper loses 50% of its strength when it has a moisture level of 14% wt%, which may be achieved by conditioning the paper at 90% relative humidity [24].

3.1.2. Basic Density. Table 3 shows the basic density of *O. abyssinica*'s at different heights. The average basic density was 0.66 ± 0.114 kg/ml, which varies between 0.362 and 1.245 g/ml. The table showed that, unlike moisture content, the basic density increased from bottom to top with values of 0.88 ± 0.25 , 0.64 ± 0.034 , and 0.42 ± 0.06 g/ml, respectively. There was no significant variation in basic density between the top, middle, and middle-to-bottom regions ($P > 0.05$). However, there was a significant difference between the top and bottom portions ($p < 0.05$) (Table 3). *Yushania alpina* (Ethiopian highland bamboo species)

recorded the basic density at the top (704.033 kg/m^3), middle (691.56 kg/m^3), and bottom (563.06 kg/m^3) parts [25]. It has a lower basic density at the top position than *O. abyssinica*'s. The current plant's basic density was lower in the center and bottom than *Y. alpina* (Table 3), indicating a higher pulping capability than highland bamboo because a low-density basic wood creates a paper with a lower beating resistance, high folding strengths, bursting, and sheet density [26, 27].

3.2. Fiber Morphology of *O. abyssinica*. An optical microscope, scanning electron microscope, and 3D optical surface profiler microscope were used to examine the surface of *O. abyssinica* fiber and its cross-sectional area. Figure 3 represents the cross-sectional area of the *O. abyssinica* bamboo block and the surface of its fiber. The cross-sectional structure of the bamboo culm is represented by many vascular bundles implanted in the scleral and parenchymal basal tissues (Figure 4(a)). Parenchymal cells are characterized by thin walls and numerous simple holes that connect them. Pits can be seen mainly on the longitudinal walls. Sclerenchyma cells, on the other hand, have thick walls. Sclerenchyma produces bundles of fibers beneath and surrounding vascular bundles in most cases. The sclerenchyma surrounding the first cycle of peripheral vascular bundles is not obstructed by interfascicular parenchyma. The *O. abyssinica* possesses the longest interwoven fiber bundle coupled with cementing-like material to give great strength, as shown by SEM and optical imaging in Figure 4.

Fiber characteristics are one of the most critical variables in determining a fiber's suitability as a pulp and paper raw material [28]. Thus, the purpose of this study component was to provide basic information on the morphological characteristics of *O. abyssinica* for the potential of pulp and paper. Lignocellulosic materials have cell wall thickness, lumen width, fiber diameter, and fiber length. These factors determine if *O. abyssinica* fiber is acceptable for paper and pulp manufacture. The fiber characteristics features of the stem section of the *O. abyssinica* fiber are shown in Table 1.

3.2.1. Fiber Length. The fiber length is the number of binding sites available on fiber to create an interwoven network of fibers. It is calculated by measuring fiber from one end to the other [10]. In this study, the fiber length of *O. abyssinica* was measured, and the main result was recorded in Figure 5. The result showed that the fiber length of *O. abyssinica* ranged from 1.32 to 3.55 mm with mean value of 2.40 ± 0.64 mm and a 95% CI value of 2.20–2.60. Therefore, the plant is classified as a long fiber plant because the mean value of fiber length is greater than 1.5 mm. The fiber length of *O. abyssinica* is comparable with the length of European red pine (2.15 mm), coniferous tree fibers (2.55 mm), jute (2.35 mm), kenaf (2.35 mm), and sisal (2.5 mm) [29, 30]. The top and bottom parts of *O. abyssinica* have significantly different ($P < 0.05$) fiber length value. However, the top and middle parts are not significantly different (Table 1). The largest mean fiber length of *O. abyssinica* was found at the bottom of the plant, with a mean of 2.59 ± 0.25 mm, while the shortest (2.23 ± 0.01 mm) was found at the top (Table 1). The overall outcome demonstrated that the plant's

TABLE 1: Fiber dimensions (top, middle, and bottom) of *Oxytenanthera abyssinica*.

Position	Statically value	Fiber length (mm)	Fiber diameter (μm)	Cell wall thickness (μm)	Fiber lumen diameter (μm)
Top	N	14	14	22	20
	$\bar{x} \pm \text{STD}$	2.23 ± 0.01^a	18.66 ± 0.22^a	2.51 ± 0.11^a	14.28 ± 0.04^a
	95% CI_L	1.91	16.87	1.86	12.71
	95% CI_U	2.54	20.45	3.16	15.79
Middle	N	14	14	20	22
	$\bar{x} \pm \text{STD}$	2.38 ± 0.02^a	21.98 ± 0.01^a	2.90 ± 1.21^b	15.54 ± 0.01^a
	95% CI_L	2.02	18.76	2.55	12.80
	95% CI_U	2.75	25.20	3.25	18.28
Bottom	N	14	14	20	20
	$\bar{x} \pm \text{STD}$	2.59 ± 0.25^b	24.87 ± 0.03^a	2.91 ± 0.24^b	17.09 ± 0.03^a
	95% CI_L	2.22	23.12	2.11	16.34
	95% CI_U	2.96	26.62	3.71	17.86
Average for a plant	N	42	42	62	62
	$\bar{x} \pm \text{STD}$	2.40 ± 0.64	21.83 ± 0.09	2.74 ± 0.03	15.63 ± 0.03
	95% CI_L	2.20	20.18	2.53	13.84
	95% CI_U	2.60	23.48	2.95	17.42

All data were examined in triplicate, and the mean value \pm SD was used. Values in the same column with the identical English alphabetical letter are not significantly different ($P \geq 0.05$) and the value in a and b are significantly difference ($P \leq 0.05$), with a confidence limit of 95%. Where: STD: standard deviation, CI_L : confidence interval lower, CI_U : confidence interval upper, N : number, \bar{x} : mean value.

TABLE 2: Fiber-derived (top, middle, and bottom) part of *Oxytenanthera abyssinica*.

Position	Statically value	Flexibility ratio	Slenderness ratio	Runkel ratio
Top	N	14	14	14
	$\bar{x} \pm \text{STD}$	0.76 ± 0.10^a	119.0 ± 0.22^a	0.35 ± 0.01^a
	95% CI_L	0.66	104.96	0.32
	95% CI_U	0.86	133.04	0.38
Middle	N	14	14	14
	$\bar{x} \pm \text{STD}$	0.71 ± 0.10^b	108.28 ± 0.12^a	0.37 ± 0.01^a
	95% CI_L	0.47	96.8	0.22
	95% CI_U	0.95	119.75	0.59
Bottom	N	14	14	14
	$\bar{x} \pm \text{STD}$	0.69 ± 0.10^b	104.14 ± 0.31^a	0.34 ± 0.01^a
	95% CI_L	0.46	80.4	0.26
	95% CI_U	0.92	127.91	0.42
Mean	N	42	42	42
	$\bar{x} \pm \text{STD}$	0.72 ± 0.10	109.98 ± 0.21	0.35 ± 0.10
	95% CI_L	0.64	104.2	0.24
	95% CI_U	0.80	115.76	0.35

All data were examined in triplicate, and the mean value \pm SD was used. Values in the same column with the identical English alphabetical letter are not significantly different ($P \geq 0.05$) and the value in a and b are significantly difference ($P \leq 0.05$), with a confidence limit of 95%. Where: STD: standard deviation, CI_L : confidence interval lower, CI_U : confidence interval upper, N : number, \bar{x} : mean value.

fiber length decreased from the base to the top. This result was comparable to the work of Wahab et al. [22]. According to their findings, the longest fiber was found near the bottom

of the *B. vulgaris* plant, while the shortest fiber was found at the top. However, according to Sharma et al. [31] and Aderounmu and Adelus [32] finding, the axial section of the bamboo species, such as *B. vulgaris* and *Dendrocalamus strictus* stems did not affect the length of the fibers. Differences in fiber lengths extracted from the axial section of *O. abyssinica* may be due to differences in internode lengths in different regions, as fiber lengths correlate with intermodal lengths [33]. Because *O. abyssinica* has long fibers greater than 1.5 mm, it can build a stronger network in the pulp [34]. For the production of paper, long fiber lengths are preferred. Long fibers produce a sheet structure that is more open and less uniform [10]. *O. abyssinica* possesses a higher fiber length when compared with a fast-growing grass called *Arundo donax* (fiber length of 1.73 mm), which has been used as an excellent raw material for handmade paper production [35]. The paper industry prefers long fiber materials for their excellent product [36].

3.2.2. Fiber Width. Figure 6 shows the fiber width of *O. abyssinica* fibers. A fiber's width or diameter is usually measured from one end to the other, usually measured across the fiber length [10]. The data showed that the average width was $21.83 \pm 0.09 \mu\text{m}$ with a 95% confidence interval of 20.18–23.48. There was a significant variation ($p < 0.05$) in fiber diameter between the top and bottom portions. Fiber width increased from the bottom ($24.87 \pm 0.03 \mu\text{m}$) to the top ($18.66 \pm 0.22 \mu\text{m}$) part of the plant. This finding is different from the work of Wahab et al. [22]. According to their result, the middle part of *B. vulgaris* had a larger fiber diameter ($18.6 \pm 0.20 \mu\text{m}$) than the top ($16.5 \pm 0.15 \mu\text{m}$) and bottom ($15.8 \pm 0.13 \mu\text{m}$) parts. *O. abyssinica* has a wider fiber width than other bamboo species, such as *B. vulgaris*

TABLE 3: Basic density and moisture content of *Oxytenanthera abyssinica*.

Location	Statically value	Mean basic density (g/ml)	Mean moisture content (%)
	<i>N</i>	6	6
Top samples (TS)	$\bar{x} \pm \text{STD}$	0.88 ± 0.30^a	8.73 ± 0.21^a
	95% CI _L	0.15	8.20
	95% CI _U	1.65	9.26
	<i>N</i>	6	6
Middle samples (MS)	$\bar{x} \pm \text{STD}$	0.64 ± 0.036^b	9.40 ± 0.32^b
	95% CI _L	0.56	8.61
	95% CI _U	0.74	10.2
	<i>N</i>	6	6
Bottom samples (BS)	$\bar{x} \pm \text{STD}$	0.42 ± 0.06^c	9.99 ± 0.21^c
	95% CI _L	0.27	9.64
	95% CI _U	0.58	10.34
	<i>N</i>	18	18
The average value	$\bar{x} \pm \text{STD}$	0.66 ± 0.11^b	9.37 ± 0.24^b
	95% CI _L	0.46	9.01
	95% CI _U	0.86	9.73

Where: STD: standard deviation, CI: confidence interval, *N*: number of sample, and \bar{x} : mean value. All data were examined more than three times, and the mean value \pm SD was used. The value in a, b and C are significantly difference ($P \leq 0.05$) with confidence limit of 95%.

(14.8 μm) [37], *Dendrocalamus giganteus* (21.34 μm) [38], *Gigantochloa apus* (14.5 μm) [38], *Melocanna baccifera* (17.1 μm) [39]. *Eucalyptus grandis* (wood plant) and Bagasse of *Saccharum officinarum* (21.4 μm) (non-wood plant) have lower fiber diameters (19.00–20.00 μm) than *O. abyssinica* [40]. The fiber width of *O. abyssinica* (21.83 μm) is greater than that of two other plants that have been tested for pulp and paper properties, such as *Melia azedarach* (hardwood plants with 13.45 μm fiber width) [41] and *Caesalpinia decapetala* (softwood plants with 18.63 μm fiber width) [42]. This gives the idea that *O. abyssinica* also can be used as an excellent raw material for pulping. The increase in fiber diameter has been connected to the many chemical and physiological changes in cell walls during the growth processes and in the vascular cambium [42]. The paper's sheet density and surface properties are affected by the diameter of the *O. abyssinica* fiber. Studies showed that paper made from fibers with a diameter of 20–40 μm frequently has high sheet density and surface properties [43].

3.2.3. Cell Wall Thickness. Figure 7 shows the cell wall thickness of *O. abyssinica* fibers. The cell wall thickness of *O. abyssinica* ranged from 1.12 to 5.255 μm , with a mean value of $2.74 \pm 0.03 \mu\text{m}$ and a 95% confidence interval of 2.53–2.95. The thickest cell wall was found at the bottom, while the thinnest was at the top. The top ($2.51 \pm 0.11 \mu\text{m}$) and middle ($2.90 \pm 1.21 \mu\text{m}$) sections have no significant ($P > 0.05$) change in their value. However, the top and bottom ($2.91 \pm 0.24 \mu\text{m}$) sections had significant values ($P < 0.05$) (Table 1). *O. abyssinica* cell wall thickness is comparable to

some wood plant cell wall thickness. It was demonstrated that *E. grandis* and *Ficus exasperate* had average cell wall thicknesses of 2.94 μm and 2.0–3.0 μm , respectively [40, 44]. The plant's cell wall thickness is, however, less than that of *Rhizophora harrissonni* (~8.8 μm) and *Rhizophora racemosa* (~9.0 μm) [45]. *Bambusa beecheyana* (6.82 μm), *B. vulgaris* (5.06 μm), *Ochlandra travancorica* (6.00 μm), and *D. asper* (5.69 μm) bamboo species have thicker cell walls than the current plant [46]. *A. donax*, which has been utilized as an excellent raw material for producing handmade paper, has a cell wall thickness of 5.36 μm . In contrast, *O. abyssinica* has a smaller thickness of 2.74 μm [35]. This demonstrated the plant's greater potential for producing pulp and paper. Plants having thinner cell walls help to make high-quality paper [47].

Figures 7 and 8 also give impotent information about the content of lignin in plant based on the colour change observed in the fiber image. The combination of the *O. abyssinica* fiber with safranin dye produces different colour observed under the fluorescence condition of specific wavelength. Safranin is able to distinguish between lignin with high and low concentrations based on changes in fluorescence emission. Although areas with little lignin illuminate green or yellow, those with high lignin content fluoresce red or orange colour. Safranin is a common azo dye used for plant microscopy, particularly as a stain for tissues that have been lignified, including the xylem. Safranin fluorescently marks the wood cell wall, causing green/yellow fluorescence in the secondary cell wall and red/orange fluorescence in the area of the middle lamella (ML) [48, 49].

3.2.4. Fiber Lumen Diameter. Figure 8 shows the fiber lumen diameter of *O. abyssinica* fibers. For several fiber sample measurements, the fiber lumen diameter of *O. abyssinica* ranged from 6.40 to 37.79 μm , with a mean value of $15.63 \pm 0.03 \mu\text{m}$ having 95% confidence limit of 13.84–17.42. The fiber lumen diameters at the top ($14.28 \pm 0.04 \mu\text{m}$), middle ($15.54 \pm 0.01 \mu\text{m}$), and bottom ($17.09 \pm 0.03 \mu\text{m}$) were all significantly different ($p \leq 0.05$). The sample with the greatest value was at the bottom, while the sample with the lowest value was at the top. The average fiber lumen diameter in the present study is larger than that of *B. vulgaris*, as reported by Wahab et al. [22]. However, the resulting value decreased from the top (2.6 ± 0.11) to the bottom (2.4 ± 0.15) compared to *O. abyssinica*. *O. abyssinica* possess a larger fiber lumen diameter than other bamboo species, such as *D. strictus* (4.33 μm), *Dendrocalamus latiflorus* (3.44 μm), *D. giganteus* (5.66 μm), *D. asper* (3.97 μm), *B. vulgaris* (3.81 μm), and *B. beecheyana* (3.55 μm) [50]. Studies showed that the bigger the fiber lumen width, the better the beating of the pulp will be [10]. During the beating process, fibers with thinner walls and broader lumens collapse more quickly and form networks strongly with one another than those with thicker walls and narrower lumens beating process [51].

The wall thickness, lumen diameter, and fiber diameter of *O. abyssinica* were generally larger at the bottom and smaller at the top. This showed that changes in fiber characteristics along the bamboo culm are caused by maturity; the older the culm segment, the better its morphological traits. Because

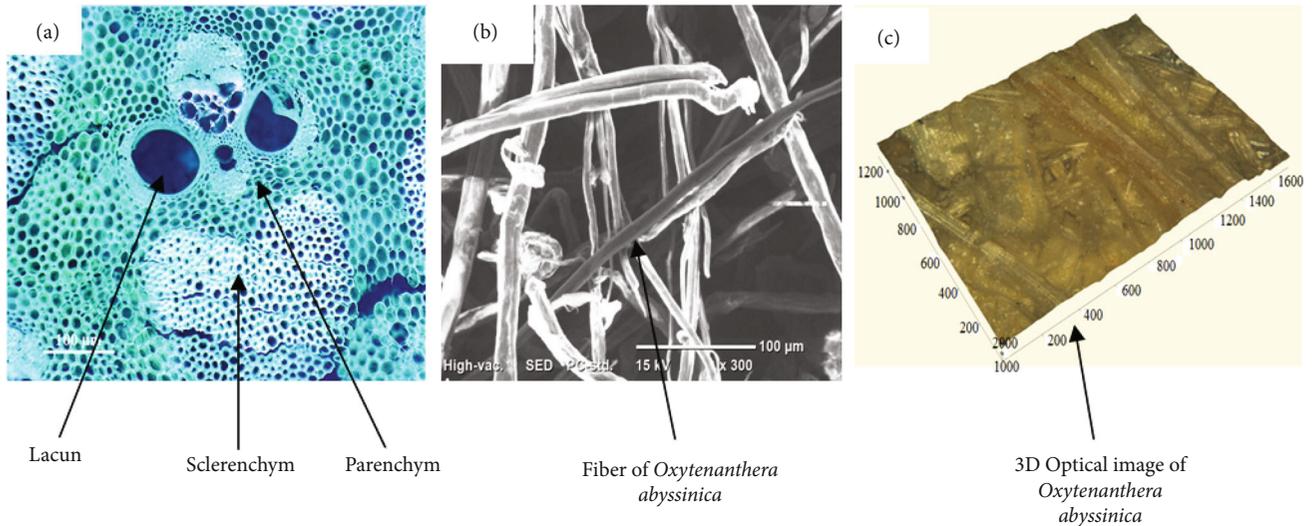


FIGURE 4: The surface of *Oxytenanthera abyssinica* fiber under an optical microscope (a, b, and c), scanning electron microscope (b).

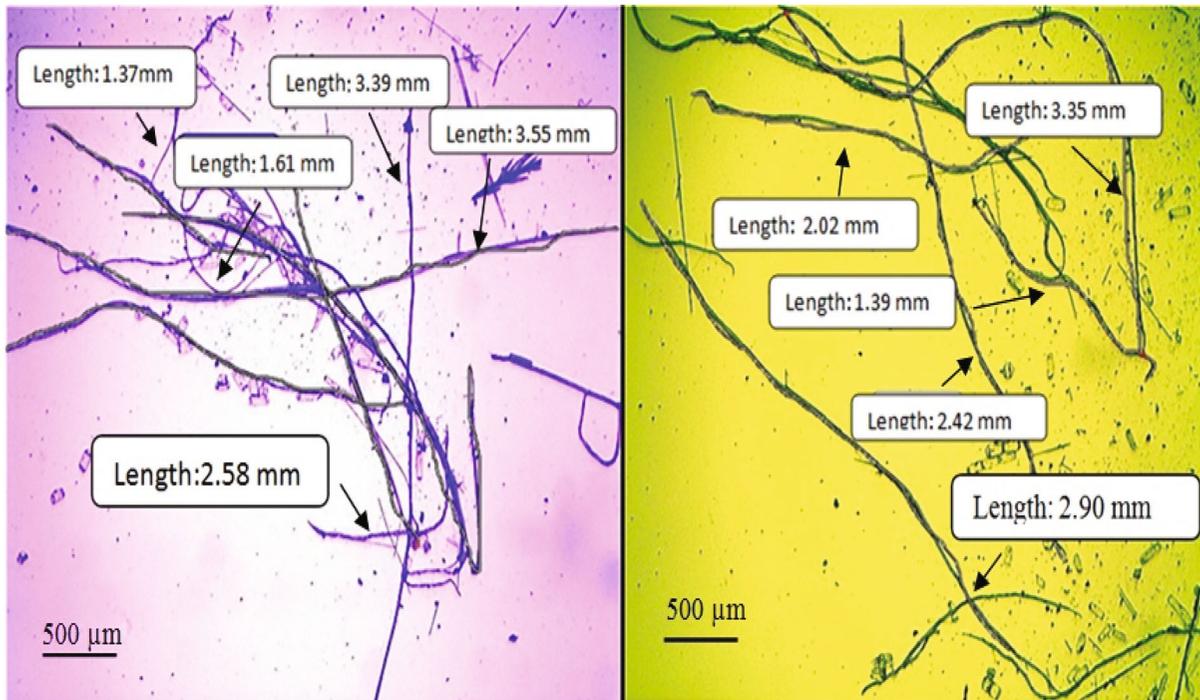


FIGURE 5: Fiber length of *Oxytenanthera abyssinica*.

the internodes at the bottom and middle of the bamboo plant spread and develop more quickly than those at the top, the fibers in those areas are often superior to those at the top [52].

3.2.5. Fiber-Derived of *O. abyssinica*

(1) *Runkel Ratio*. The Runkel ratio of wood fiber is one of the wood characteristics essential for pulp and papermaking [53]. Table 2 showed that *O. abyssinica* had an average Runkel ratio value of 0.35 ± 0.01 with a 95% confidence interval of 0.24–0.35. The maximum Runkel ratio was found in the middle of

the plant (0.37 ± 0.01), and the minimum was found in the bottom (0.34 ± 0.01). No significant difference ($P \leq 0.05$) between the top, middle, and bottom regions.

A high pulp yield will be obtained if the Runkel ratio is less than one [54]. Low Runkel ratio fibers are often thin-walled and have greater strength properties, inter-fiber bonding, and greater conformability [55, 56]. The Runkel ratio of fibers from all portions of *O. abyssinica*'s is less than 1, thus, it is satisfactory to recommend the plant for producing flexible pulp and paper with good mechanical properties [37, 57].

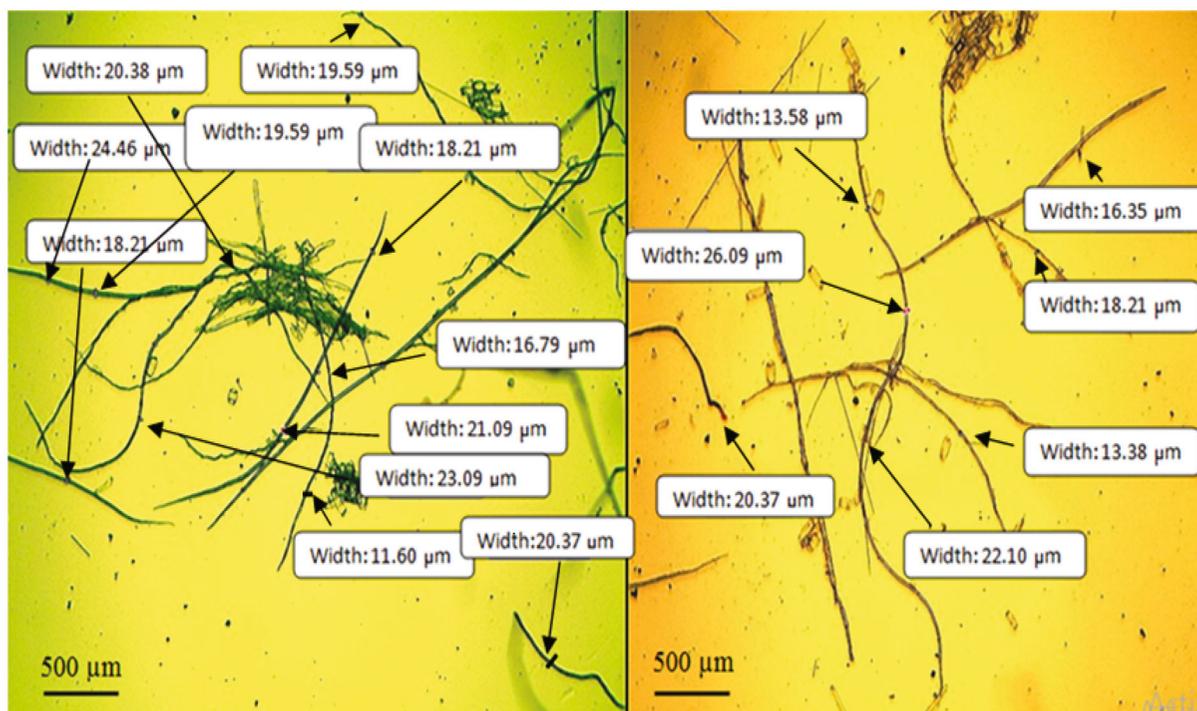


FIGURE 6: Fiber width of *Oxytenanthera abyssinica*.

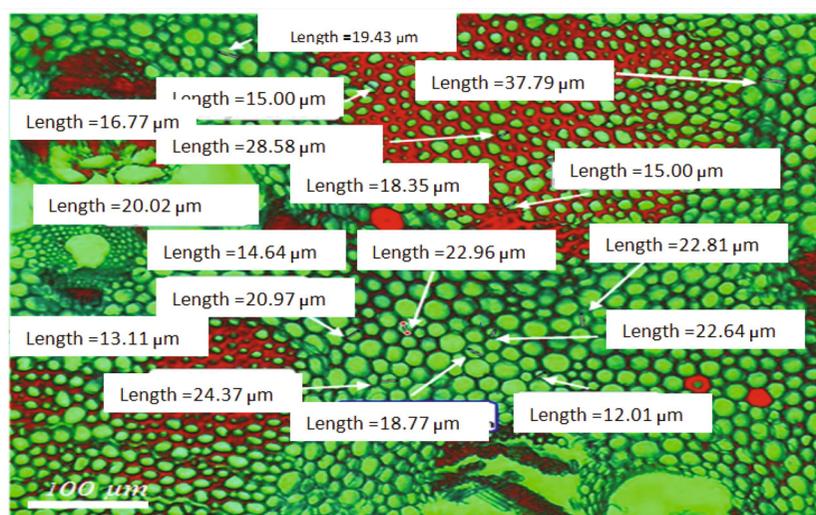


FIGURE 7: Cell wall thickness of *Oxytenanthera abyssinica*.

(2) *Flexibility Ratios*. The flexibility ratio indicates how easily fibers link and, as a result, how strong the tensile and bursting strength are generated [58]. Table 2 showed that *O. abyssinica* had an average flexibility ratio value of 0.72 ± 0.01 with a 95% confidence interval of 0.64–0.80. There was a significant variation in the flexibility ratio between the central and lower parts of *O. abyssinica* ($P \leq 0.05$). The results also showed that it decreased from the top (0.76 ± 0.1) to the bottom (0.69 ± 0.1) along the culm. The flexibility ratios of *O. abyssinica* fibers were between 0.50 and 0.75 in all portions of the plant; this showed that the plant possesses elastic properties [43]. Elastic fibers commonly recommend for manufacturing

packing papers [40, 59]. According to studies, the higher the fiber length-to-width ratio, the more flexible the fiber is and the more likely it is to produce a well-bonded paper [60].

(3) *Slenderness Ratio*. Table 2 showed that *O. abyssinica* had a slenderness ratio mean value of 109.98 ± 0.21 with a 95% confidence interval of 104.2–115.76. The difference in the slenderness ratio was significant ($P \leq 0.05$) between the bottom (104.14 ± 0.31), the middle (108.28 ± 0.12), and the top (119.00 ± 0.22) sections. The slenderness decreased from the top (119.00 ± 0.22) to the bottom (104.14 ± 0.31) along the culm. The fibers from all parts of *O. abyssinica* have a

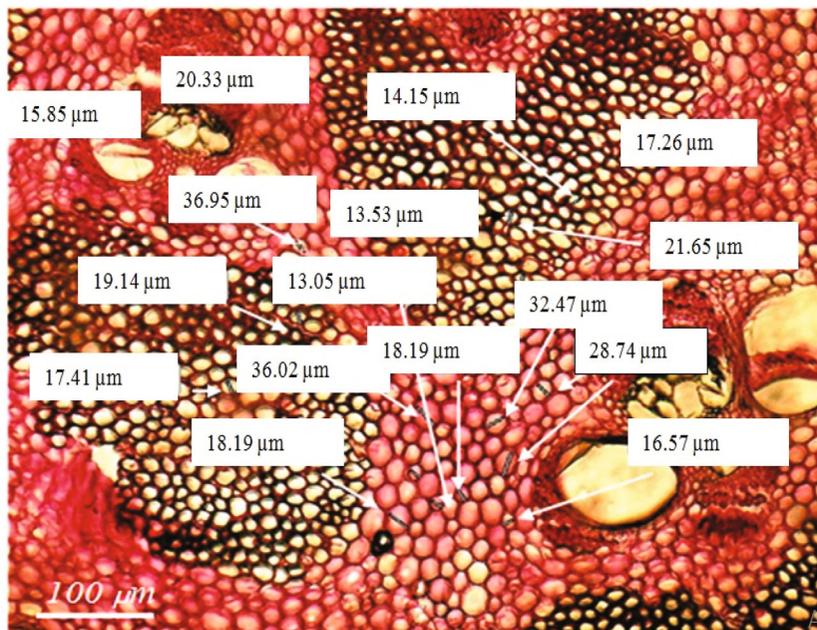


FIGURE 8: Fiber lumen diameter of *Oxytenanthera abyssinica*.

slenderness ratio greater than 70. This suggests that the fibers create high-quality sheets for packing and other applications, as mentioned in [58].

3.2.6. Comparison of Fiber Characteristics with Other Bamboo Species. In comparison to two well-known bamboo species such as green bamboo and Moso bamboo, *O. abyssinica* has a longer fiber length (2.40 mm) and diameter (21.83 mm), as well as a smaller Runkel ratio (0.350) (Figure 9) [61]. However, the Runkel ratio of green bamboo and Moso bamboo were greater than one (>1), with values of 2.96 and 4.53, respectively. This indicates that the papermaking potential of *O. abyssinica* is higher than the two bamboo species because a low Runkel ratio means larger fiber lumen width and a thin fiber wall. A thin fiber wall is desirable for high-quality, strong, and well-formed paper. Moreover, the beating of pulp, which involves liquid penetration into gaps within the fiber, is positively influenced by large lumen size. Thus, fibers having a high Runkel ratio are less flexible, stiffer, and produce bulkier, low-bounded-area paper [62]. For the production of high-quality paper, long fiber lengths are preferred. Long fibers produce a sheet structure that is more drainable and less uniform. The flexibility, tensile, and burst strength of paper are all improved by thin cell walls [62, 63]. This also indicates that *O. abyssinica* has a higher potential for papermaking properties than the other two bamboo species (Figure 9).

3.3. Chemical Composition. The results of the proximate chemical analysis of *O. abyssinica* at 1, 2, and 3 years of age were reported in Table 4. The average cellulose, hemicelluloses, and lignin content in 3-year-old *O. abyssinica* were 49.26 ± 0.13 wt%, 21.31 ± 0.15 wt%, and 20.63 ± 0.12 wt%, respectively. The percentage of cellulose content increases when the ages of *O. abyssinica* become older. The mean cel-

lulose content at ages 1, 2, and 3 were 49.26 ± 0.13 wt%, 48.87 ± 0.15 wt%, and 48.21 ± 0.15 wt%, respectively. The mean cellulose content of the 3-year-old, 2-year-old, and 1-year-old *O. abyssinica* samples differed significantly ($P < 0.05$). However, the average cellulose content of the 2- and 1-year-old bamboo samples did not differ significantly. The current studies have shown that the cellulose content of *O. abyssinica* is higher than that of all hardwoods and softwood plants [64].

The hemicellulose content of *O. abyssinica* in the ages of 1, 2, and 3 years old are 21.31 ± 0.15 wt%, 23.17 ± 0.11 wt%, and 21.05 ± 0.22 wt%, respectively. The hemicellulose content of the 3-year-old and 2-year-old bamboo samples did not differ significantly ($P \geq 0.05$). However, the content of the 1- and 2-year-old and 1- and 3-year-old bamboo samples differed significantly ($P \leq 0.05$). As can be seen in Table 3, the hemicellulose content was highest at 2 years old compared to *O. abyssinica* at ages 1 and 3. The hemicellulose content in the current study was lower than the softwood content [64]. The average percentages of lignin content in *O. abyssinica* at ages 1, 2, and 3 are 20.63 ± 0.12 wt%, 23.54 ± 0.33 wt%, and 23.03 ± 0.24 wt%, respectively. There was a significant difference in lignin content between bamboo samples aged 3 and 2, and 3 and 1 years. Like hemicellulose, *O. abyssinica* has the highest lignin content at 2 years of age. The average percentages of extractives and ash content at ages 1, 2, and 3 are (6.8 ± 0.15 wt%, 7.16 ± 0.15 wt%, and 7.67 ± 0.15 wt%) and (2.645 ± 0.11 wt%, 4.505 ± 0.17 wt%, and 6.63 ± 0.153 wt%) in a respective manner. As the plant becomes older, the percentage of ash and extractive content decrease.

The content of cellulose, hemicelluloses, and lignin of the *O. abyssinica* is in the range of hardwood content. The lignin content in the plant in this study showed a lower value than softwood and hardwood [64] when compared to wood;

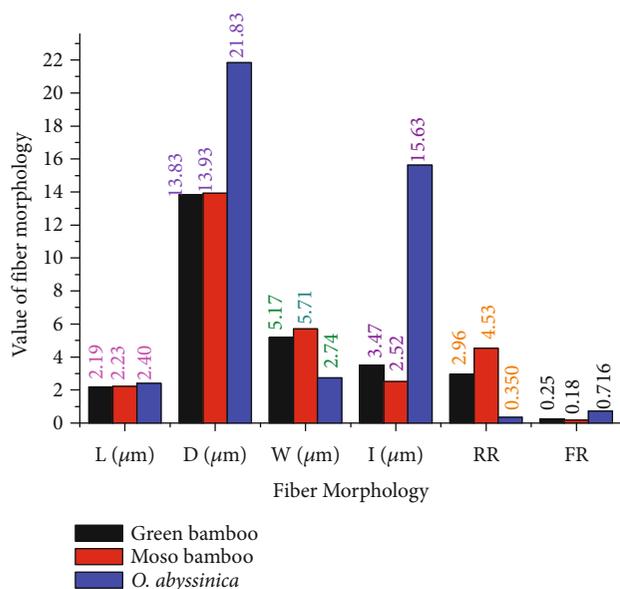


FIGURE 9: Fiber morphological comparison between *Oxytenanthera abyssinica*, green bamboo, Moso bamboo. Where L: fiber length; D: fiber diameter; W: cell wall thickness; l: fiber lumen diameter; RR: Runkel ratio; and FR: flexibility ratio.

this showed a more efficient delignification under the same cooking conditions. This means that to achieve a suitable kappa value, *O. abyssinica* would require milder pulping conditions (lower chemical charges and temperatures) than hardwoods and softwoods, which could reduce chemical consumption and energy usage.

Three aged *O. abyssinica* bamboo species has got higher solubility in hot water (11.67 ± 0.15 wt%), cold water (9.56 ± 0.15 wt%), alcohol benzene (4.51 ± 0.15 wt%), and 1% NaOH solubility (20.4 ± 0.15) than 2- and 1-year aged bamboo species. There was no significant difference ($P > 0.05$) in the value of cold water and alcohol-benzene solubility between 1-, 2-, and 3-year samples (Table 4). *O. abyssinica* has a greater alcohol-benzene solubility of 4.51 ± 0.153 wt% than green bamboo (*Dendrocalamopsis oldhami*) species ($3.3\text{--}3.9$ wt%) [66], which is used for pulp and papermaking. This indicated that *O. abyssinica* has a higher concentration of salts, low molecular weight carbohydrates, non-volatile hydrocarbons, phytosterols, resins, lipids, waxes, and other water-soluble substances than green bamboo. This information confirmed that the study plant has higher pulp and paper potential. Solubility in 1% NaOH solution of *O. abyssinica* was (20.4 ± 0.15 wt%). The value is higher than the other solubility such as cold water solubility (9.56 ± 0.15 wt%), hot water solubility (11.67 ± 0.15 wt%), and alcohol-benzene solubility (4.51 ± 0.15 wt%). Solubility in a 1% NaOH solution indicates the degree of fungal decay or degradation of wood. 1% NaOH solubility also indicates the degree of solubility of extractive chemicals, some lignin, and low molecular weight hemicellulose [65].

The alpha-cellulose content of 3-year aged *O. abyssinica* was (48.8 ± 0.23 wt%) (Table 5). This value is higher than the seven Indonesian bamboo species studied by Maulana et al. [65]. According to their study, the alpha-cellulose con-

tent of Ando bamboo and Ampel bamboo is less than 40 wt%, and the alpha-cellulose content of Senbiran bamboo and Kunin bamboo is 40–45 wt%. Begun bamboo, Hitam bamboo, and Tari bamboo have high alpha-cellulose content greater than 45 wt%. This showed that *O. abyssinica* could have higher pulp and paper production potential than Indonesian bamboo species because alpha-cellulose positively affects the pulp yield [66]. Further research has been conducted to link the amount of cellulose to pulp yields. For instance, according to Dillner et al. [67], cellulose contents and kraft pulp yields of *Eucalyptus globulus* wood were closely connected, and Wallis et al. [68] demonstrated that eucalypt wood samples with a high cellulose content produced more pulp.

3.4. Chemical Composition Comparison to the Other Pulping Raw Materials. The chemical composition of other bamboo species in the current study was compared in Table 5. The table showed that the cellulose content (49.26 wt%) of 3-year-old *O. abyssinica* was higher than that of other bamboo species such as *Dendrocalamus brandisii* (47.24 wt%) [69], *Bambusa blumeana* (40.3–45.1 wt%) [70], *Bambusa tuldoidea* (35.2 wt%) [71, 38], *Gigantochloa levis* (33.8 wt%) [71], *Passiflora edulis* (44.64 wt%) [39], and *Daphniphyllum oldhami* (47.1 wt%) [66]. This showed that *O. abyssinica* has a great potential for pulp yield and papermaking properties than the mentioned bamboo species. As shown in Table 5, *O. abyssinica* has a greater cellulose content (49.56%) and a smaller lignin content (20.63%) than various agricultural pulping raw materials like wheat straw, barley straw, corn stalk, jute, and sorghum stalk. Regarding hollo-cellulose content, except for oil palm fronds, the other agricultural pulping raw materials mentioned in the table have a smaller value than the current study. This showed that the lowland Ethiopian bamboo (*O. abyssinica*) plants collected from the western part of Ethiopia might have a higher potential as a raw material for pulp and papermaking than agricultural waste materials.

Many researchers have claimed that cellulose concentration and pulp production are directly related. Kiaei et al. [63] found a positive link between cellulose concentration and pulp quality. High cellulose concentration yields high pulp yield, according to Biermann [77]. The hemicelluloses content of *O. abyssinica* is higher than that of *D. brandisii* [69], *B. vulgaris* [76], *Phyllostachys edulis* [39], and *Daphniphyllum oldhami* [76]. This also explains why the plant has a higher potential for pulp yield than the plant's mentioned above. The amount of hemicellulose has a positive relationship with pulp yield. Higher hemicellulose values, for example, lead to increased pulp yield and paper strength (mainly fold strength, burst, and tensile) [77]. The current study's lignin content of *O. abyssinica* exhibited the lowest lignin content among the other 10 bamboo species listed in table except *B. blumeana*. This provides information on how suitable the plant is for pulp and paper production because the amount of lignin negatively affects pulp yield [78, 40]. Lignin causes fiber-to-fiber bonding to be delayed in the paper, and all of its properties have a negative impact on paper production; as a result, high-quality papers are made from lignin-

TABLE 4: Chemical composition value of *Oxytenanthera abyssinica* at 1-, 2-, and 3-year aged.

Age of the plant	Statically value	Cellulose (wt%)	Lignin (wt%)	Hemicellulose (wt%)	Ash (wt%)	Extractives (wt%)	Hot water (wt%)	1% NaOH (wt%)	Solubility Alcohol benzene (wt%)	Cold water (wt%)
3 years	N	3	3	3	3	3	3	3	3	3
	$\bar{x} \pm \text{STD}$	49.26 ± 0.13^a	20.63 ± 0.120	21.31 ± 0.15^a	2.64 ± 0.11	6.8 ± 0.15^a	11.67 ± 0.15^a	20.4 ± 0.15	4.51 ± 0.15^a	9.56 ± 0.15^a
	95% CI _L	47.39	18.73	16.06	2.09	5.18	9.25	16.89	1.02	3.30
	95% CI _U	51.13	22.52	26.55	3.19	8.41	14.08	23.90	7.99	15.82
2 years	N	3	3	3	3	3	3	3	3	3
	$\bar{x} \pm \text{STD}$	48.87 ± 0.21^b	23.54 ± 0.33^b	23.17 ± 0.11^b	4.50 ± 0.17	7.16 ± 0.153^b	9.11 ± 0.93^b	17.42 ± 1.03	4.40 ± 0.71^a	9.49 ± 0.71^a
	95% CI _L	44.21	21.21	20.03	3.78	6.28	5.92	11.33	2.46	6.48
	95% CI _U	53.53	25.87	26.30	5.23	8.03	12.30	23.50	6.34	12.50
1 year	N	3	3	3	3	3	3	3	3	3
	$\bar{x} \pm \text{STD}$	48.21 ± 0.12^b	23.03 ± 0.24^b	21.05 ± 0.22^a	6.62 ± 0.15	7.67 ± 0.153^c	7.54 ± 0.76^c	14.56 ± 1.42	4.21 ± 0.55^a	9.01 ± 0.71^a
	95% CI _L	44.17	19.00	17.43	1.68	3.89	1.86	10.19	4.14	7.07
	95% CI _U	52.25	27.00	24.66	11.57	11.44	13.21	18.92	4.27	10.32

All data were examined in triplicate, and the mean value \pm standard deviation was used. Values in the same column with the identical English alphabetical letter are not significantly different ($P \geq 0.05$) and the value in a and b are significantly difference ($P \leq 0.05$), with a confidence limit of 95%. STD: standard deviation, CI_L: confidence interval lower, CI_U: confidence interval upper, N: number, \bar{x} : mean value.

TABLE 5: Chemical composition of *Oxytenanthera abyssinica* to other bamboo species.

Samples	Alpha-cellulose	Holocellulose (wt%)	Lignin (wt%)	Hemicellulose (wt%)	Cellulose (wt%)	Ash (wt%)	Reference
<i>Oxytenanthera abyssinica</i>	48.8 ± 0.23	70.57 ± 0.28	20.63 ± 0.12	21.31 ± 0.15	49.26 ± 0.13	3.30 ± 0.11	This study
<i>Phyllostachys heterocyclus</i>	—	—	26.1	—	49.1	1.3	[38]
<i>Phyllostachys nigra</i>	—	—	23.8	—	42.3	2.0	[38]
<i>Phyllostachys reticulata</i>	—	—	25.3	—	25.3	1.9	[38]
<i>Bambusa blumeana</i>	—	65.70	20.50	—	40.3	—	[70]
<i>Bambusa tuldoidea</i>	—	67.20	25.5	32.0	35.2	3.70	[71]
<i>Dendrocalamus asper</i>	48.60	68.25	25.27	—	—	2.13	[38]
<i>Dendrocalamus brandisii</i>	—	—	23.84	23.85	47.24	1.37	[69]
<i>Dendrocalamus giganteus</i>	46.88	65.96	23.85	—	—	3.70	[38]
<i>Gigantochloa apus</i>	47.56	63.23	22.41	—	—	6.09	[38]
<i>Gigantochloa brang</i>	51.18	79.94	24.83	—	51.58	1.25	[73]
<i>Gigantochloa brang</i> (outer part)	56.94	80.05	38.75	—	—	0.78	[74]
<i>Gigantochloa levis</i>	33.81	85.08	26.50	—	33.8	1.30	[72]
<i>Gigantochloa levis</i> (outer part)	36.96	89.80	35.98	—	—	1.19	[74]
<i>Gigantochloa robusta</i>	44.36	56.81	23.86	—	—	1.66	[38]
<i>Goniothalamus scortechinii</i>	46.87	74.62	32.55	—	46.87	2.84	[73]
<i>Goniothalamus scortechinii</i> (outer part)	61.31	75.35	43.68	—	—	1.50	[74]
<i>Melocanna baccifera</i>	—	73.5	25.2	21.1	52.78	2.45	[39]
Corn cobs	—	—	15	31	50.5	—	[75]
Jute	—	17.0	24.6	—	—	—	[7]
Wheat straw	—	15–20	—	20–25	33–40	—	[76]
Barley straw	—	14–19	27–38	—	31–45	—	[7]
Barley straw	—	66.01	19.47	—	—	10.97	[7]
Cornstalk	—	69.92	18.16	—	—	7.75	[7]
Sorghum stalks	—	11	25	—	27	—	[7]
Rice straw	—	18	18	24	32.1	15–20	[7]
Oil palm fronds	—	83.00	21.00	—	—	2.5	[7]
Sugarcane bagasse	—	32–48	23–32	—	19–24	—	[75]
Olive husk	—	23.6	48.4	—	24	—	[75]

free fibers [79]. Extractives from raw materials are undesirable because they can interfere with the pulping and bleaching processes. High extractive concentration, according to Ates et al. [43], will indicate low pulp production as well as increased chemical usage in pulping and bleaching [80]. Different soil types, age and maturity levels, and raw material sources could explain the variances in cellulose, lignin, hemicelluloses, ash, and extractives reported in this bamboo species (Table 5). Genetics, age, location, growth conditions, anatomic structure, and plant maturity level also influence the chemical composition of lignocellulosic materials [81].

4. Conclusion

In this study, we examined the chemical composition and fiber characteristics of *O. abyssinica* to assess its compatibility for pulp and paper production. The result indicated that the average Runkel ratio was 0.350 (<1) with 95% CI of 0.24–0.35, the slenderness ratio was 109.98 (>70) with 95%

CI of 104.2–115.76, and the fiber length was 2.40 (>1.5) with 95% CI of 2.20–2.60. The experimental data showed that the amount of cellulose in the culm increases and the amount of ash and extractive decreases as it becomes older (from 1 year to 3 years). However, the highest amount of lignin and hemicellulose content was found in 2 years, followed by 3 and 1 year. The average chemical content of the three years aged *O. abyssinica* was 49.26 ± 0.13 wt% (95% CI of 47.39–51.13), 21.31 ± 0.15 wt% (95% CI of 16.06–26.55), and 20.63 ± 0.12 wt% (95% CI of 18.73–22.52) for cellulose, hemicellulose, and lignin contents, respectively. The bulk densities of *O. abyssinica* were 660 kg/m³, and its moisture content was 9.37 wt%. Thus, the above data showed that *O. abyssinica* could have pulp and papermaking potential. The 3-year bottom part of the plant is preferable to the middle and top part of 1-year and 2-year aged *O. abyssinica*. The current study employed samples up to 3 years old; therefore, future research should focus on samples older than 3 years because a plant's characteristics rely on its age, culm height,

environment (whether it is farmed or natural), type of soil it grows in, and the area in which it is found.

Data Availability

All data generated or analyzed during this study are included in this paper.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

L. A. W. carried out the experiments and produced the paper in a journal fashion. He also created the necessary tables and figures for the document. A. B. and R. K. B. drafted, revised, and rewrote the paper, as well as provided advice throughout the development of the concept, with M. G. T. proofreading the manuscript. The published version of the manuscript has been read and approved by all authors.

Acknowledgments

The authors thank Addis Ababa Science and Technology University (AASTU) to give a chance to do this research work and Ethiopian Environmental and Forest Research Institute Wood Technology Research Center for fiber characterization. A preprint has previously been published in Research Square under the following link <https://www.researchsquare.com/article/rs-2429210/v1A> [82].

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