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Production and characterization of cellulose from the branch and leaves of *Gigantochloa atter* (Kawayang kayali)

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Abstract

Gigantochloa atter (*Kawayang kayali*) branch and leaves were extracted and characterized as an alternative source to produce cellulose. Delignification and bleaching process were carried out to produce cellulose. The produced cellulose was then characterized through spectroscopic, morphological and several physical properties. The amount of cellulose present in *G. atter* branch and leaves were 12.5521 ± 0.2655% and 8.3037 ± 0.4733%, respectively, which both appeared to be in amorphous white powder. Meanwhile, result in *t*- test indicted that there is a significant difference between the two percent yields of cellulose between *G. atter* samples. The cellulose produced were both soluble in cuprammonium hydroxide and partially soluble in NaOH, implying the absence of noncellulosic components. FT-IR analysis also confirmed through spectral match that the samples produced are both cellulose. In addition, the absence of C=O stretch and aromatic ring of C=C in the spectra implies the removal of lignin for both *G. atter* samples. Both samples did not impart a red violet stain upon treatment of phloroglucinol – HCl solution, which also implies that no lignin was left in the samples. The surface structure of the product shows a clear separation of fibers before and after chemical treatments. Overall, the results suggested that both *G. atter* samples are a potential source of cellulose.

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Introduction

Gigantochloa atter (Kawayang kayali), belongs to the *Gigantochloa* genus and subfamily of *Bambusoideae* under *Gramineae* Family. *G. atter* is known for its availability and bulk productivity. Its woody parts like culms have also massive utilization in building constructions and other living tools. Other than cotton, bamboo has also a greater amount of fibers. Hence, worldwide research about bamboo had been carried out to study its structure, properties and extraction methods of its fiber (Kaur *et al.*, 2013).

Based on the studies involving other varieties of bamboo, part of the components that can be extracted from bamboo is cellulose. It is the main component in plants in which it determines the structure of a cell wall. Other than cellulose, lignocellulosic component such as, pectin, lignin and hemicellulose may also be found, which is located between the cellulose microfilaments of the cell wall (Li, 2004).

Culms contain cellulose, hemicellulose and lignin, which amount to over 90% of the total mass (Li, 2004). On the dry weight basis, bamboo fibers in culms have the highest cellulose percentage with 40%. Thus, it makes bamboo plants rich in cellulose that can be used for the wide production for fabrication. In which, culms are particularly used in fabrications and leaving the branches and the leaves as waste materials which can also be used as a good source for cellulose.

Bamboo have been widely used to industrial and domestic applications due to the advances in processing technology and increased market demand. As a low-cost source of cellulose, G. *atter* offers a great potential for the mass production of cellulose fibers. Different extraction procedures to plant sources have been developed, which includes delignification and bleaching. (Lavanya *et al.*, 2011)

On this basis, this study is conceptualized to focus on the extraction and characterization of cellulose fiber from the branch and leaves of *Gigantochloa atter*. Hence, the main objective of this study is to produce and characterize the cellulose fiber from the branch and leaves of *Gigantochloa atter*. In addition, this study also aimed to compare which part of the Bamboo plant- the branch and leaves has the higher percentage cellulose yield and to compare the different physical properties of the produced cellulose from branch and leaves of *G. atter* possesses.

Materials and methods

Sample and Reagents Preparation

About one (1) sack of green branch and leaves that are found in the aerial part of *G. atter* were randomly collected at Terresa Heights Eastside Barangay, Isabela City, Basilan, Philippines. It was then identified as *Gigantochloa atter* (Kawayang kayali) by the Department of Environment and Natural Resources, Region IX. The reagents that were being used for this study were analytical- grade and were directly used without further purifications.

Bamboo Cellulose Production

The collected bamboo branch and leaves were washed thoroughly using distilled water, air- dried for a week, then pulverized using a blender and a grinder, respectively to convert into powder. The powdered samples were then oven- dried at 105°C for 3 hours to dry. The sample was then labeled and kept in resealable plastic bags for later use.

About 2.0 grams of dried and pulverized samples were placed in separate 250ml beakers and treated with 20ml of 10% v/v nitric acid. The beakers containing the resulting mixture were then heated at 70 – 90°C for 1 hour. The samples were then cooled at room temperature, filtered using a Buchner funnel and Whatman 2V filter paper, then washed with distilled water until the filtrate became colorless. The residues were then placed in an aluminum tray and dried in the oven at 80 - 110°C for an hour until complete dryness.

The dried samples from the *G. atter* samples were placed in separate 250ml beakers and treated with 1.0 M sodium hydroxide in a 1g:20ml sample:solvent ratio. Resulting mixtures were then heated in a hot plate at 70 – 90°C for one hour with constant stirring. After which, the mixtures were removed from the hot

plate and cooled for 15 minutes. The cooled samples were then filtered using Buchner funnel and Whatman 2V filter paper and washed with distilled water until the filtrate became colorless. The residues were placed in an aluminum tray and dried for one hour in the oven at 80 – 110°C. The dried samples were cooled for fifteen minutes, then weighed.

The dried samples were then placed in separate 250ml beakers then treated with 5% sodium hypochlorite in a 1g:20ml sample:solvent ratio. The mixtures were then heated in a hot plate at 70 – 90°C with constant stirring for one hour. The mixtures were then removed from the hot plate and cooled for 15 minutes. After cooling, the samples were filtered using Buchner funnel and Whatman 2V filter paper, washed with distilled water until the filtrate becomes colorless. The residues were then placed in the oven for drying at 100 - 105°C for one hour. The dried samples were cooled, weighed, and recorded as cellulose (Mora *et al.*, 2008). The extracted cellulose samples were placed in a small closed glass container and it was placed in dessicator for later use.

The determination of cellulose percent yield was calculated using [Equation 1]:

$$\text{Percent Yield} = \frac{m_{\text{Cellulose}}}{m_{\text{Sample}}} \times 100\% \quad \text{[Equation 1]}$$

where $m_{cellulose}$ is the weight recorded in 3.3 (i.e. amount of cellulose after bleaching) and m_{sample} is the mass of raw *G. atter* samples used. Five trials were done for this part.

Characterization of Produced Cellulose

Different physicochemical tests such as solubility tests, FT-IR (Shimadzu IRAffinity-1S) and chemical test for lignin (phloroglucinol- HCl test) were used to determine the degree of purity of cellulose produced from branch and leaves of *G. atter*.

Morphological Analysis

The pinch of the raw material and extracted cellulose fibers were placed on separate petri dish and were soaked with distilled water. Then, the fibers were placed on separate slides and observed under a photomicrograph (Eco line by Motic) at a 400x magnification. Morphological tests were done to describe the structure of the raw material used with its respective produced cellulose (Hughes *et al.*, 2013).

Results and discussion

Determination of Percentage Yield of Cellulose from G. atter Branch and Leaves

The cellulose from the *G. atter* samples were extracted using the strong acid, strong base and a bleaching agent- sodium hypochlorite. The amount of cellulose obtained from *G. atter* samples were summarized in Table 1.

Table 1. Percentage Yield of Cellulose from *G. atter* Bamboo Samples.

Sample	Weight of Sample	Weight of Sample after chemical treatment (g)			Percent Yield (%)
		HNO_3	NaOH	NaClO	
Branch	2.3587 ± 0.0217	1.5948 ± 0.0492	0.8356 ± 0.0598	0.2960 ± 0.0051	12.5521 ± 0.2655
Leaves	2.3543 ± 0.0470	1.5618 ± 0.0558	0.6582 ± 0.0442	0.1955 ± 0.0122	8.3037 ± 0.4733

The *G. atter* branch contains greater amount of cellulose (12.5521 ± 0.2655) compared to *G. atter* leaves (8.3037 ± 0.4733). This is in accordance with literature as a bamboo branch comprises of more fibers in which the cellulose was predominantly present. Thus, it gives more strength and helps the bamboo plant to remain stiff and strong.

The reaction of acid on both pulverized samples is caused not only by the decomposition of nitric acid at a specific temperature but also with the dissolution of an organic material present like pectin. Meanwhile, the addition of sodium hydroxide enables the separation of other unwanted materials present in the sample such as lignin and hemicellulose. (Supranto *et al.*, 2015) The addition of sodium hypochlorite has the capacity to decrease the carbonyl groups present in the samples, leaving white pure cellulose. Thus, hypochlorite lessens the impurities that were not removed the previous treatments. (Henniges and Potthast, 2009)

Meanwhile, based on Table 2 indicates that student's *t*- test result calculated the *t* value of the *G. atter*

samples at 17.43 in which it is greater than the value of t_{crit}. at 2.45. Thus, it implies that the null hypothesis was rejected and can be concluded that there is a significant difference between the two percent yields of cellulose obtained from the *G. atter* branch and leaves. Therefore, the amount of cellulose extracted from *G. atter* branch is significantly different compared to *G. atter* leaves.

Table 2. Comparison of Cellulose Extracted from *G. atter* Branch and Leaves Between the Percent Yields.

Parameters	Sample	
	Branch	Leaves
Average ± Std. dev.	12.5540 ± 0.2655	8.3040 ± 0.4733
t Stat	17.4318	
P(T<=t) two-tail	2.2853E-06	
t Critical two-tail	2.4469	
Implication	Significant	

Characterization of Cellulose

A. Solubility Test

The solubility of cellulose produced was done to determine if the extracted cellulose were soluble or insoluble with different solvents, which is summarized in Table 3.

Table 3. Solubility Data Result of Cellulose.

Cellulose	Solvents Used			
	Distilled Water	Ethanol	Sodium Hydroxide	Cuprammonium Hydroxide
Branch	-	-	+	++
Leaves	-	-	+	++

Legend: (-) not soluble, (+) partially soluble, (++) completely soluble

Hydroxyl groups and polar groups such as distilled water and ethanol exhibit little interaction with cellulose. In which, cellulose contains a high molecular weight and bulky structure, which it compromises hydrogen bonding between -OH groups on adjacent chains. Thus, cellulose is insoluble in distilled water and ethanol. In 5% percent NaOH, meanwhile, chain degradation due to alkaline treatment occurs upon dissolution, turning color of the mixture to yellow as cellulose becomes partially soluble with sodium hydroxide.

Meanwhile, Schweitzer's reagent or cuprammonium hydroxide is a highly alkaline medium, in which the hydroxyl groups of cellulose are deprotonated ($OH \rightarrow O^-$) in the presence of Cu(II), thus the $Cu(NH_3)_n(OH)_2$ was reacted to the deprotonated hydroxyl groups and form chelate complexes. The solubility of cellulose is dependent only on the copper concentration and the ability of cellulose to neutralize the cuprammonium to the extent of using completely the hydroxyl group arising from the dissociation. Thus, it can be deduced that cellulose was completely soluble, which is congruence with the result obtained in Table 2 (Runckel, 1942).

B. FT-IR Analysis of G. atter Samples

Fig. 1A shows the broad peak intensity band, of the cellulose from branch, at 3359.09cm^{-1} that was attributed to O-H stretching band. The fingerprint region peak at 1636.63cm^{-1} corresponds as Fiber-OH, the water associated with cellulose and conjugated C-O. The water absorbed in the cellulose molecules was hard to extract due to the cellulose-water interaction. At peak ranges of 1316.44cm^{-1}, 1157.31cm^{-1} and 1105.23cm^{-1} corresponds the pyranose ring, in which it splits out the water from the process to form a glycosidic bond (Muller, 2008).

Meanwhile, Fig. 1B shows that the peak at 3350cm^{-1} indicates the O-H stretch, while the -OH in the literature was boarder compare to the produced cellulose due to free hydroxyl group present (Kennedy *et al.*, 2009). Meanwhile, the peak intensity at 2903.88cm^{-1} indicates C-H stretching, which means that the hydrogen bond was increased after the chemical processes (Mora *et al.*, 2008). The fingerprint region on the spectrum shows that the peak at 1641.45cm^{-1} corresponds to Fiber-OH, which means that the water was being absorbed in the cellulose molecules due to the less interaction of water in cellulose molecule (Muller, 2008). At the peak of 1425.42cm^{-1} corresponds to the aromatic ring of C-C, also with the C-H rocking at peaks of 1367.55cm^{-1} and 1321.26cm^{-1}. The peaks of 1157.31cm^{-1}, 1105.23cm^{-1} and 1049.29cm^{-1} indicated that an ether functional group C-O-C that corresponds to pyranose skeletal ring, which is caused by splitting out the

water from the process to form a glycosidic bond (Kontturi, 2015).

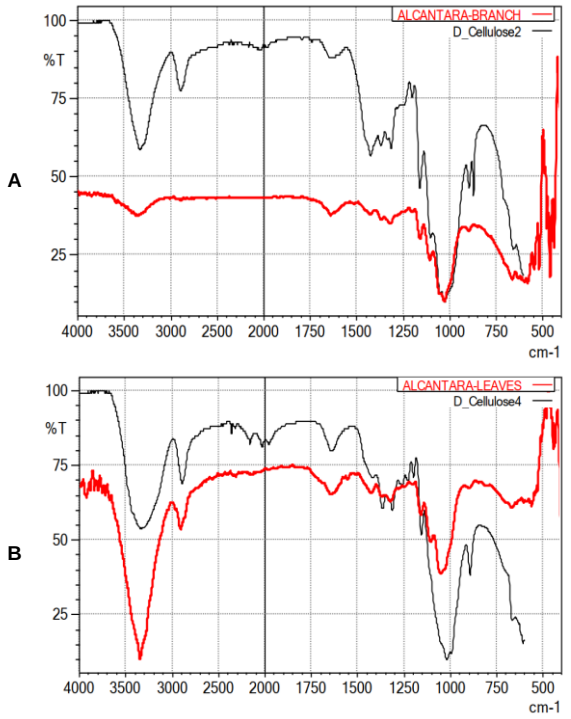

Fig. 1. Infrared Spectra of Produced Cellulose from *G. atter* A) Branch and B) Leaves.

C. Test for Lignin and Morphological Analysis

The raw materials and the extracted cellulose of the both *G. atter* branch and leaves had undergone a test for lignin and it was also observed under the photomicrograph as presented in Fig. 2.

For the test of lignin, the raw material for both samples, upon subjecting to phloroglucinol – HCl solution imparted of red-violet color thus, indicating that there is a presence of lignin in both samples. The aldehyde end groups of lignin appeared to react with phloroglucinol-HCl to impart a red-violet color (Tao *et al.*, 2009). Meanwhile, the extracted cellulose from *G. atter* branch leaves showed that the structure had completely separated from the non-cellulosic treatments after the chemical treatments. The cellulose fibers were completely seen and there is no appearance of red - violet color thus, suggesting that the lignin was completely dissolved after treatment by the strong base.

Meanwhile, the morphological test shows the structure of the *G. atter* samples before and after chemical treatments is also shown in Fig. 2. The image of raw material from *G. atter* branch and leaves showed that the compacted fibers were not separated before the chemical treatments. After the delignification and bleaching process, the extracted cellulose from *G. atter* branch and leaves showed the separation of fibers thus, the lignocellulosic component was dissolved during the chemical treatments. Its surface was not only less dense but also clearer compared to the raw materials used.

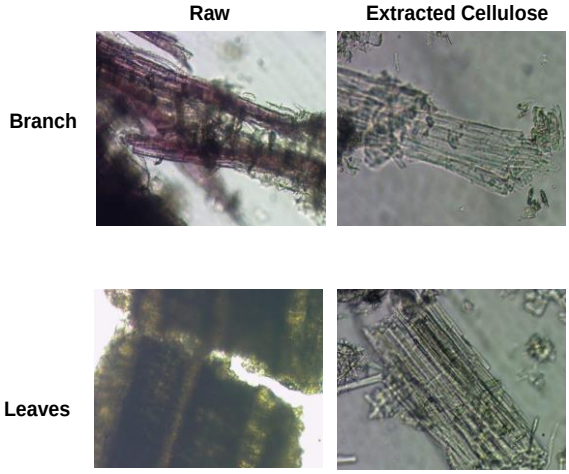

Fig. 2. Photomicrograph Images of Raw *G. atter* Branch and Leaves, and the Extracted Cellulose from *G. atter* Branch and Leaves (400x).

Conclusion and recommendations

The separation of noncellulosic components from the cellulose fiber was achieved using nitric acid, sodium hydroxide and with sodium hypochlorite treatments, producing a white amorphous powder with percentage cellulose yield of 12.5521 ± 0.2655% and 8.3037 ± 0.4733% from *G. atter* branch and leaves, respectively.

The extracted cellulose from *G. atter* samples were characterized by solubility test and FT-IR analysis. Cellulose from *G. atter* branch and leaves were both soluble in cuprammonium hydroxide, caused by the deprotonation of hydroxyl groups in the presence of Cu(II) ions, implying that the obtained cellulose was completely separated from the other noncellulosic components. The spectra in the FT-IR analysis confirmed the removal of hemicellulose and lignin as the characteristic peaks of lignocellulosic materials were absent. Finally, the spectrum for both *G. atter*

samples after spectral match showed that it was similar to cellulose.

Meanwhile, the fibers of both raw materials of *G. atter* samples were packed together and the fibers were not fragmented. For the extracted cellulose, however, the structures of both *G. atter* samples were separated showing the surface of cellulose fiber. The structure of raw materials containing the noncellulosic component imparted a red-violet stain after treatment with HNO_3, NaOH and NaClO, while the extracted cellulose which was separated from the noncellulosic component showing the absence of a red-violet color stains.

Based from the results obtained, it is recommended to produce cellulose from the other parts of *G. atter* as well as other bamboo species. In addition, the concentration, temperature, time and pH in the extraction process of cellulose should be varied so as to optimize the cellulose percentage yield of the said material.

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