Biocellulose isolated from the waste of pinecone flower (Pinus merkusii Jungh Et De Vriese)

S E Lusiana¹, M M Aisiyah¹, U Z ‘Uyunin¹, Z D Nasihin¹, A Srihardyastutie¹, M F Rahman³ and M Masruri¹*

¹Chemistry Department, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia
Jl. Veteran 65145 Malang

*Corresponding author’s email: masruri@ub.ac.id

Abstract. A bio-cellulose isolated from pinecone flower waste has been isolated by two type procedures. The first method is commonly used in industrial protocol. It is undergone via delignification and bleaching processes. In the delignification process provide a black-liquid of lignin waste, meanwhile the bleaching process afford neutralized clear liquid. The bio-cellulose is the main product. On the hand, the second method is initiated by soxhlet extraction using three different solvents i.e. n-hexane, ethanol and toluene, respectively. The extraction process produces organic hexane extract, ethanol extract, and toluene extract. All these extract have different appearance in color and prospective. Further delignification and bleaching process provide black lignin waste and neutralized liquid waste. The bio-cellulose still also become a major product.

1. Introduction
Bio-cellulose is a cellulose derived from biological sources, such as plants, animals, and microorganism [1]. The cellulose is classified as polysaccharide that is composed of the monomers of glucose. To be precise is D-glucopyranose which is the glucose that has a cyclic structure (Figure 1). The connection inter-glucose molecule is attached by glycoside bond. It occurs between hydroxyl group at C4 and hydroxyl-group attached in C1 by dehydrating of water molecule in enzymatic reaction. In biological sample, cellulose mixes to the other chemicals inside the cell such as water, lipid, lignin, and protein. And to isolate a pure cellulose, the procedure has to be able to separate cleanly the other matrix from cellulose [2].
Figure 1. Molecular structure of cellulose. Glycoside bond connect the glucose monomers.

The common procedure applies a base such as sodium hydroxide and potassium hydroxide solution to the mixture for dissolving lignin, protein, chlorophyll and other water soluble material. A black-soluble chemical mostly contain lignin generally extracted, and cellulose is located as residue. The bleaching process oxidized the other material that are soluble in basic conditions [3]. However, in term of “green chemistry”, this procedure disposes many organic chemicals that composes in the cell, such as terpenoid, fatty acid, phenolic, tannin, and alkaloid group of the secondary metabolites [4].

Figure 2. Two type methods for cellulose isolation from pinecone flower waste.

2. Experiment section

2.1. Sample and chemicals

The pinecone flower waste is collected from local industrial pine forest in Malang, Indonesia. Pine species is Pinus merkusii Jungh Et De Vriese and the taxonomy is previous reported by the same group [5, 6]. The sample is further washed and dried in air for 2 days before grinding to powder. Meanwhile the chemicals used for research includes sodium hydroxide (Merck), sodium hypochlorite (Sigma), hydrochloric acid (Smart Lab), iron trichloride (Merck), gallic acid (Merck), formic acid (Merck), acetic acid (Merck), n-hexane (Merck), ethanol (Merck), toluene (Smart Lab), and potassium bromide (Merck).
2.2. Instruments
The instruments used for the research includes FTIR spectrophotometer (Shimadzu FTIR), laboratory grinder, analytical balance (Mettler), and mechanical stirrer.

2.3. Experiment procedure
The schematic of procedure for isolation of cellulose from pinecone flower waste is depicted in Figure 2. There are 2 type procedure for isolation. Namely procedure 1 and procedure 2.

2.3.1. Procedure 1. A dried powder of pinecone flower waste (50 g) in Erlenmeyer-1000 mL is added 500 mL sodium hydroxide solution 6.0%. This mixture is heated at 70 °C and stirred for 4 hours. Then, it is filtered off and the solid part is undergone washing with water to neutral. The neutral solid is further undergone bleaching process by addition 300 mL of sodium hypochlorite solution 6%. It is stirred for 2 h at 70 °C and the solid part is separated from the liquid, and is further bleached for 6 time, consecutively. The product is washed with water exhaustively to afford neutral cellulose. Then, it is separated by filtration, and the solid product is cellulose.

2.3.2. Procedure 2
2.3.2.1. Soxhlet extraction. A dried powder of pinecone flower waste (150 g) in the timble is set to the soxhlet apparatus. The n-hexane (650 mL) is added and the process is undertaken in several cycles for 6 hours. The liquid extract is evaporated under reduced pressure to afford a brown-black concentrated extract of hexane. The solid residue retained in the soxhlet timble is further undergone extraction using toluene as solvent (650 mL). The process is proceeded for several cycle for 6 hours. After concentration in rotary evaporator affords the toluene extract as a concentrated of black-brown extract of toluene. The residue retained in the timble is further extracted using ethanol (650 mL). It is also proceeded for several cycles for 6 hours. The ethanol extract is afforded as a clear-brown liquid after concentration in vacuo using rotary evaporator. The solid residue in the soxhlet timble is undergone further process for cellulose isolation.

2.3.2.2. Cellulose isolation. The dried of solid residue in above procedure (50 g) is basified with 500 mL of sodium hydroxide solution 6.0%. This mixture is heated at 70 °C and stirred for 4 hours. Then, it is washed with water until neutral pH. The solid residue is further added with 300 mL of sodium hypochlorite 6.0%, heated at 70 °C and stirred for 2 hours. The product is separated and the solid residue is added with sodium hypochlorite. The process is repeated for 4 times to afford a white cellulose. Then, it is washed until neutral pH and dried at room temperature.

![Figure 3. Cellulose results is produced following 2 type procedure.](image-url)
3. Result and discussion
The cellulose composes a part of cell life in plants. It sticks and mixes with other chemicals contains in the cell wall. Protein, secondary metabolite products, and other biological matrix has to be opened and separated from cellulose. For example, lignocellulose binds strongly to cellulose, and cleavage the lignin from cellulose requires a strong base. Including other polar of phenolic groups such as tannin, phenylpropanoid compounds and the alkaloids [7].

Grinding process of sample pinecone flower waste can increase the surface area interaction between reagent and chemicals (Figure 3). The direct delignification of the pinecone flower powder affords a black aqueous lignin after filtration. The residue, further bleaching process can afford cellulose in coarse size. A yellowish-white cellulose is afforded.

The second procedure give a better result. Some organic solvent extracts derived from n-hexane, toluene, and ethanol is afforded. It is predicted secondary metabolite composes these extract. Generally, non polar group such as sesquiterpene steroid, and triterpenoid class of terpenoid composed the n-hexane extract. Meanwhile, toluene generally applied for tannin, lignin, and other phenolic groups extraction process. And, ethanol can extract all of fatty acid and polar groups of compound include protein and polyketide [8]. The quantity of cellulose isolated from that procedure is reported in Table 1. The first procedure is able to provide better yield. Cellulose is isolated in 44.06%. Meanwhile the second procedure give a slightly lower yield (38.45%). However, cellulose appearance and potential additional product given in the second procedure opens the more prospective.

Table 1. Yield of cellulose isolated.

| Method | Cellulose isolated (%) | Cellulose appearance | Potential additional products |
|--------|------------------------|----------------------|------------------------------|
| 1      | 44.06%                 | coarse powder white-brown | Lignin                      |
| 2      | 38.45%                 | fine powder white-brown  | Lignin n-Hexane extract (6.9%) Toluene extract (8.6%) Ethanol extract (5.1%) |

Molecular characterization of the cellulose isolated is undertaken using FTIR spectrophotometry (Figure 4 and Figure 5). Finger printing analysis is methodologically applied for qualitative analysis. The similarity of bands wavelength of the sample is compared to the band wavelength from reference, especially in finger printing region. This region correlates to the similarity in the structure and composition [9].

Table 2. Functional group and fingerprint bands of cellulose.

| Characterized of functional groups | Bands absorption wavelength (1/cm) |
|-----------------------------------|-----------------------------------|
|                                   | Cellulose 1 | Cellulose 2 | Commercial cellulose |
| Hydroxyl (O-H)                    | 3400.0      | 3420.0      | 3391.0               |
| Alkyl (CH₃; CH₂-)                  |             |             |                        |
| Stretching band                    | 2901.0      | 2909.0      | 2906.7                |
| Bending band                       | 1267.9-1509.9 | 1267.9-1510.9 | 1300.0-1430.0         |
| Carbonyl (C=O)                     |             |             | 1760.0                |
| C=C alkene                         | 1634.4      | 1640.1      | 1654.4-1635.2         |
| C-O-C ether group                  | 1130.7-1161.8 | 1130.6-1161.8 | 1061.7-1162.4         |
Figure 4. The FTIR spectra of the commercial cellulose as the reference.

The hydroxyl-functional group is detected in about 3400 cm\(^{-1}\). The reference spectra from the commercial cellulose come up at 3391 cm\(^{-1}\). In addition, the corresponding band stretching for this group, C-O, is recorded in between 1000 cm\(^{-1}\) and 1265 cm\(^{-1}\). For the alkyl stretching band, such as C-H and H-C-H, is detected in 2900 cm\(^{-1}\) in all sample cellulose. And also their bending bands (1200-1500 cm\(^{-1}\)). Carbon-carbon double bond group is recorded in all samples (1634-1654 cm\(^{-1}\)). However, the carbonyl group is only detected in commercial cellulose (1760 cm\(^{-1}\)) (Table 2). This finding indicate the high purity and no structural changing of cellulose isolated from pinecone flower waste.
4. Conclusion
To summarized, cellulose can be isolated in high yield from pinecone flower waste. The two type procedure can be applied to afford cellulose. The second procedure can generate another potency for prospecting of secondary metabolite extracted using n-hexane, toluene and ethanol as solvents. Further stage has to be undertaken to analyze the composition these extract, and also conversion of the isolated cellulose into prospective products or starting materials.

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