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Original Paper

The Optimization Of Cellulose Content In Tobacco Stems (*Nicotiana tabaccum* L.) With Acid Extraction Method And Alkaline Extraction Method

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***Abstract* -- Tobacco stems are the agricultural waste of tobacco crops that have the potential to be utilized. Generally, only the leaves of tobacco stems are used without proper handling. Tobacco stems have the potential as a source of cellulose because they have a relatively high content of cellulose. The use of proper methods plays an important role in extracting cellulose on tobacco stems. The research aims to determine the efficiency levels of cellulose extract from tobacco stems (Nicotiana tabaccum L.) with acid extraction methods and alkaline extraction. The research used a complete randomized design with two trials. The results showed that the alkaline method produced the best results with respect to the rendemen testing parameters, inlcluding water content, hemicellulose content, cellulose content, lignin content and FTIR spectroscopy. According to the yield test, the best moisture, hemicellulose content, cellulose content and lignin content were found in the alkaline extraction with a concentration of 12%, 43.63%; 7.54% water content; 14.33% hemicellulose content; 46.17% cellulose content; and 2.83% lignin content. FTIR spectroscopy test shows that all treatments have important and specific function groups on cellulose such as – OH, C-H, and-CH_2.**

Keywords: tobacco stems, cellulose, extraction acid, alkaline extraction.

I. INTRODUCTION

Tobacco is one of the commodities belonging to the seasonal crop of plantation. Statistical data of Indonesian commodity tobacco plantations stated that tobacco production in 2016 reached 126,728 tons. As of 2019, the production increased even further by reaching 197,250 tons [19]. However, the use of tobacco for cigarettes that only require tobacco leaves will leave parts of the stem and roots [15]. Tobacco stems after harvest period are allowed to dry out without further effective processing, leading to agricultural waste. Tobacco stems have the potential as a source of cellulose because they have a relatively high content of cellulose [16].

The highest cellulose content in tobacco plants is found in tobacco stems that reach 35-40% of dried tobacco stems [10, 14, 17]. It is congruent with the research results from [6] demonstrating that the tobacco stems have cellulose of 56.10%, Hemicellulose by 22.44%, and lignin 15.11%. Cellulose has a chemical structure ($C_6H_{10}O_5$) which is composed of a polymer with a straight chain of 1.4 ß-D glucose. Cellulose can be obtained by extracting the plant stem. Extraction process can be done by acid extraction method and alkaline extraction method.

Previous works have highlighted the values concerning the high outcome of tobacco stem waste in Indonesia and the relatively high content of cellulose in tobacco stems, providing the potential of tobacco stems as a source of cellulose to increase the use value of waste [18]. The use of proper methods plays an important role in extracting cellulose on tobacco stems. Therefore, this study will examine cellulose content in tobacco stems (*Nicotiana tabaccum L.*) resulting from acid extraction methods and alkaline extraction.

II. RESEARCH METHODOLOGY

A. Research Site and Time

This research was conducted at the Agroindustry Management Laboratory of the Faculty of Agricultural Technology and the Chemical Laboratory of the Faculty of Pharmacy, Jember University. This research was conducted from January to June 2020.

B. Materials and Equipment

The materials used in this study were tobacco stems, aquades, 95% technical ethanol, 96% technical H2SO4, technical NaOH, and technical HCl. The equipment included analytical scales, blender, oven, FTIR instrument, waterbath, measuring cup, erlenmeyer, Beaker glass, magnetic stirer, measuring pipette, pipette drops, stirring rod, funnels, aluminum plate, hotplate, Desiccator, and sieve 60 mesh as well as 80 mesh.

C. Research Design

This research uses a Completely Randomized Design (CRD) with 2 repetitions. The research plan can be seen in Table 1.

Table 1. Research plan

Extraction Methods	Type of Treatment		
	P1	P2	P3
A	AP1	AP2	AP3
B	BP1	BP2	BP3

Explanation:
AP1: Acid extraction method with 7% concentration
AP2: Acid extraction method with 9% concentration
AP3: Acid extraction method with 12% concentration
BP1: Alkaline extraction method with 7% concentration
BP2: Alkaline extraction method with 9% concentration
BP3: Alkaline extraction method with 12% concentration

D. Research Procedure

The study consisted of extraction by acidic methods and extraction by alkaline methods. The research procedure can be seen in Figure 1 and Figure 2.

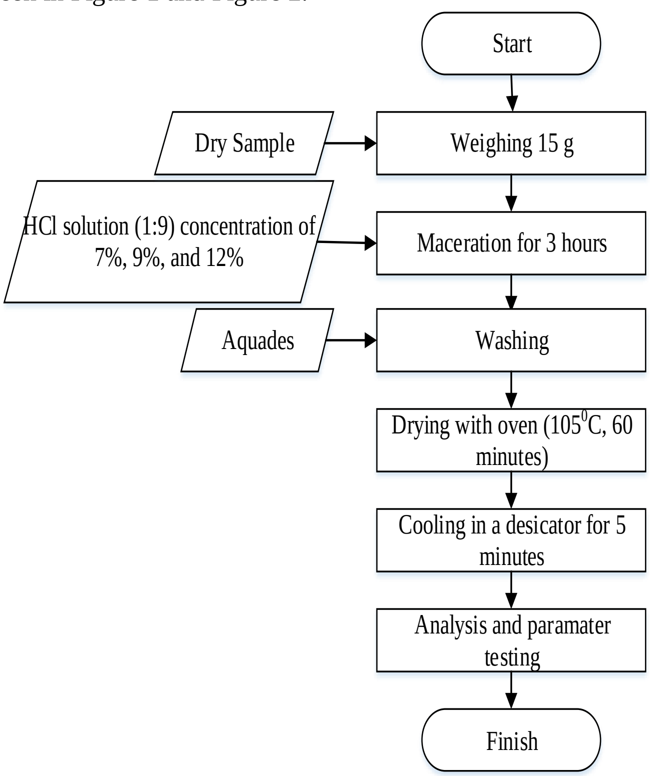

Figure 1. Procedure of Acid Extraction Method

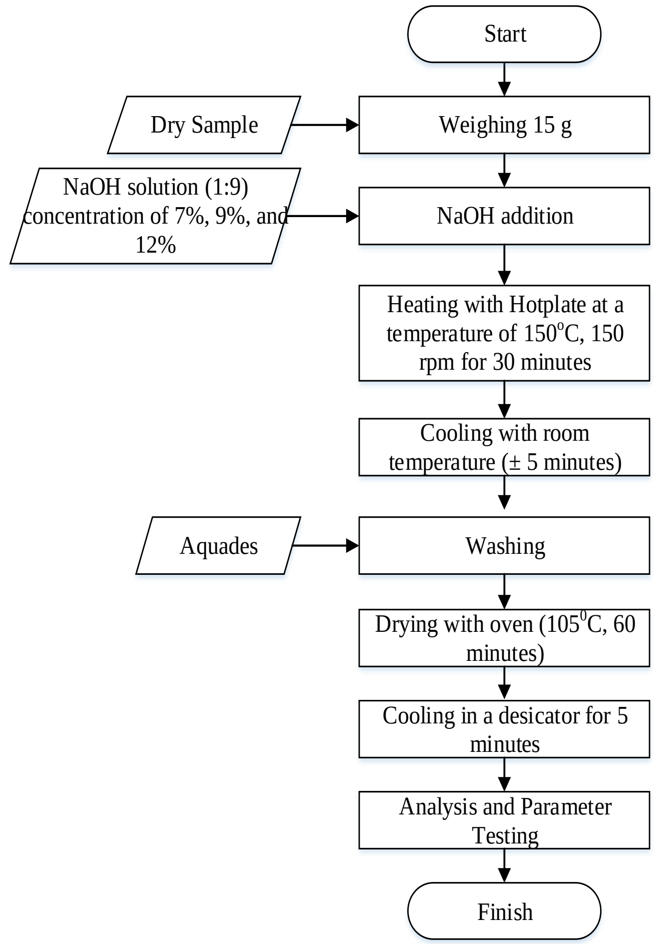

Figure 2. Procedure of Base Extraction Method

E. Analysis Procedure

Yield Value [1]

The yield value was obtained by comparing the amount of initial dry material used with the amount of final dry material obtained.

$$\text{Yield Value : } \frac{Final\ Weight}{Initial\ Weight} x\ 100\%$$

Explanation:
Initial weight: Weight of the sample used in the dry Condition (g)
Final weight: The weight of the yield obtained at last weighing (g)

Water Content [1, 20]

Water content testing was performed using the thermogravimetric method.

$$\text{Ka (\%wb)} = \frac{[C+A]-[C+B]}{[C+A]-C} x\ 100\%$$

Description :

C = Cup weight (g)
A = Sample weight (g)
B = Weight of sample after roasting (g)

Lignocellulose Analysis with the Chesson-Datta Method [8]

The analysis of Lignocellulose (lignin, Hemicellulose, and cellulose) used the Chesson-Datta Method and through calculations on existing equations. Chesson-Datta method was applied in analyzing sulfuric acid (H_2SO_4).

$$\text{Hemicellulose} = \frac{b-c}{a} x\ 100\%$$

Description:
a = The initial weight of the tobacco stem cellulose sample (g)
b = The dry weight of the sample residue heated with aquades (g)
c = Residual weight after being heated with H_2SO_4 1 N (g)

$$\text{Cellulose} = \frac{c-d}{a} x\ 100\%$$

Description:
a = The initial weight of the tobacco stem cellulose sample (g)
c = Residual weight after being heated with 1N H_2SO_4 (g)
d = Residual weight after being soaked with 72% H_2SO_4 and heated with H_2SO_4 1 N (g)

$$\text{Lignin} = \frac{d-e}{a} x\ 100\%$$

Description:
a = The initial weight of the tobacco stem cellulose sample (g)
d = Residual weight after immersion with 72% H_2SO_4 and heated with H_2SO_4 1 N (g)
e = Weight of the final residue after drying (g)

F. FTIR Spectroscopy Analysis

FTIR spectroscopy analysis is aimed at identifying polymers and chemical bonds in test sample fibers. This analysis is based on the wavelength and intensity that absorbs infrared radiation so that the vibration occurring in each group of functions represents the number of waves.

G. Data analysis

Research data were processed using Microsoft Excel and ANOVA method processing using SPSS (Statistical Package for the social science) application at 0.05 real level to know the effect of of measured parameters. If there is a difference between the average treatment, a test is carried out using the Dunn's Multiple Range Test (DMRT) at a 5% signification level. The data for the test results and the calculations are presented in the form of *spider web* chart.

III. RESULTS AND DISCUSSION

A. Water Content

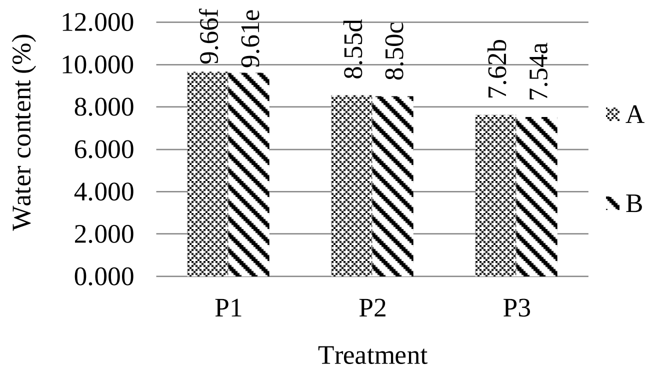

Figure 3. Results of water content value of the acid extraction method and the alkaline extraction method

Based on ANOVA's results, the influence of the extraction and concentration methods on the water content was distinct from real with the value of Sig. <0.05, the test of Dunn's Multiple Range Test (DMRT) was carried out. Water content on acid extraction methods decreased with the lowest water content at a 12% acid concentration of 7.62%. Water content for alkaline extraction methods also decreased so that the concentration of alkaline solution 12% obtained the lowest water content of 7.54%. The increase in the concentration of acid solution and the alkaline solution used to cause a decrease in the water content in the sample due to decreased water content caused by water molecules contained in evaporation of materials during the drying process.

B. Yield Value

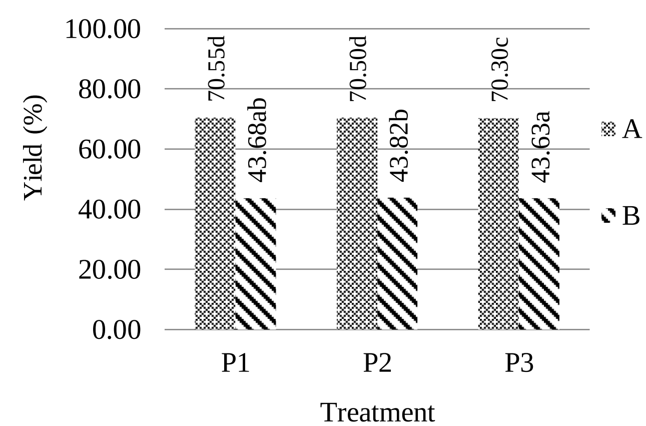

Figure 4. Yield value of acid extraction method and alkalin extraction method

Based on ANOVA's results, the influence of the extraction method and concentration on the yield result marked a significant different as evinced by Sig. <0.05. Again, Dunu's Multiple Range Test (DMRT) was at play. The yield value in the acid extraction method decreased, so that the acid concentration of 7% produced the highest yield value of 70.55%. Alkaline yield value at 9% alkaline solution concentration had the highest value of 43.82%. The decrease in yield resulted from the breakdown of the hemicellulose and lignin component structures and the decrease in water content in tobacco stems. The broken component structure will undergo hydrolysis into simple compounds that are more soluble in water during the washing process [5].

C. Hemicellulose Content

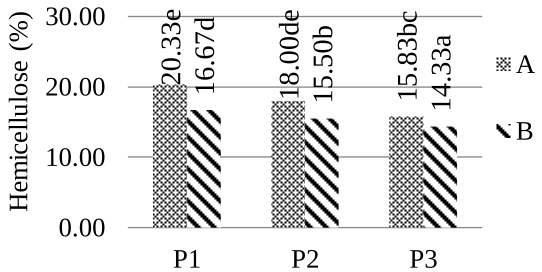

Figure 5. The hemicellulose content value from acid extraction method and base extraction method

Based on ANOVA's results that the influence of the extraction and concentration methods on the results of Hemicellulose content differ by the obtained value of the Sig. < 0.05, so it is followed by the test of Duncan's Multiple Range Test (DMRT). Hemicellulose content of acid extraction method and alkaline extraction decreased with a 12% acid solution concentration by 15.83%. Results of Hemicellulose content for alkaline extraction method also decreased so that the lowest alkaline solution concentration with 12% concentration by 14.33%. This decrease in Hemicellulose content is due to its largely soft (amorphous) structure, making it easily hydrolyzed by alkaline and acids.

The process of hydrolysis occurring ions OH-on NaOH will break the bonds of endo-β-xylanase, α-glucoronidase, β-xylosidase and α-arabinofuranosidase resulting in hydrolyzed Hemicellulose into simple water-soluble sugars. Hemicellulose has an irregular (amorphous) region and is easily hydrolyzed in acids. Hydrolyzed hemicellulose will be its monomer, D-glucose, D-manosa, D-arabioasa and D-xylose. Hemicellulose which has been hydrolyzed by adding HCl to its constituent monomers will easily dissolve in water.

D. Cellulose Content

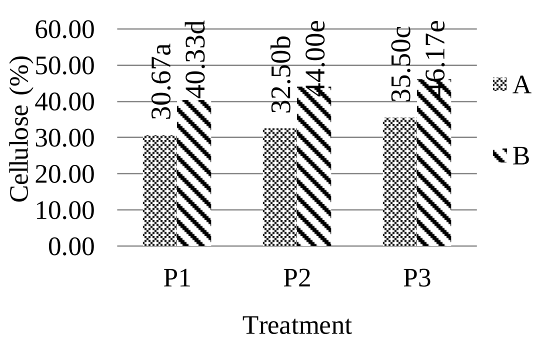

Figure 6. The cellulose content value from acid extraction method and alkaline extraction method

Based on ANOVA results, the effect of extraction method on cellulose content was significantly different as demonstrated by Sig value <0.05, so the analysis further involved Duncan's Multiple Range Test (DMRT). The level of cellulose in acid extraction method increased, while at the same time marked by the highest cellulose content at 12% and 35.50% acid concentration. The cellulose content from the base extraction method increased, so the concentration of 12% alkaline solutions obtained the highest content of cellulose at 46.17%.

The increase of acid solution and alkaline solution by increasing the solution concentration does not trigger hydrolysis in cellulose. Hydrolysis occurring in Hemicellulose and lignin is higher than that in cellulose. The ongoing process has hydrolysed other compounds such as hemicellulose and lignin, which eventually increases cellulose content [4]. The crystalline and amorphous regions which have the task of protecting glucose units in damaged cellulose will cause cellulose to decompose into glucose so that it can dissolve easily in water. Therefore, higher cellulose content will prevent damage [13].

E. Lignin content

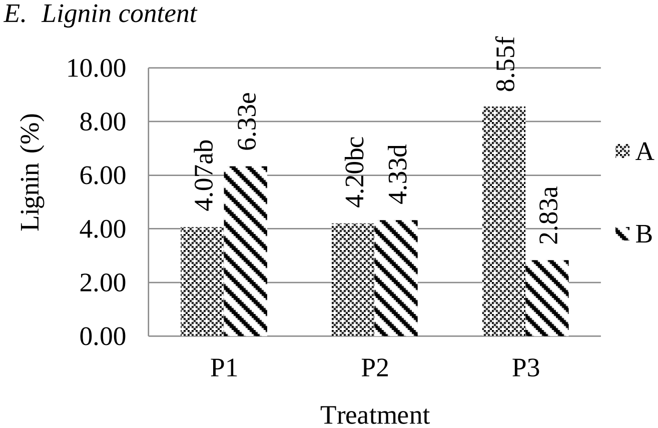

Figure 7. The Lignin content from the acid extraction method and alkaline extraction method

Based on ANOVA results, different extraction method and concentration generated different results on lignin content, demonstrated by Sig value <0.05, Duncan's Multiple Range Test (DMRT) was operative. Lignin content from acid extraction method is increased due to the lowest lignin concentrations at 7% and 4.07% acid concentration. Lignin content from alkaline extraction method decreased, which implied that concentration of base solution 12 obtained lowest lignin rate by 2.83%.

According to [9], the higher concentrations of alkaline solutions will decrease lignin content. O^-Ion of NaOH will break the bonds of ligning structure of lignin, and Na^+ ions will bind lignin and form a phenolic salt. The polar phenolic salts will become easily soluble during the washing process [11]. Increased lignin content occur when 12% acid solution concentrations is used, typically due to H^+ ions at high concentration of HCl which is not able to disconnect lignin bonds optimally. According to [8], an increase in lignin contents occurs because the lignin contained in the sample is deposited once high acid solution concentration is added. This affects the accumulation of molecular weights and causes an increase in lignin molecular weights.

E. The Best Extraction Method Results

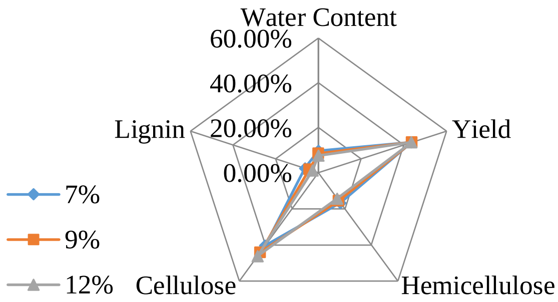

Figure 8. Spider web presenting water content, hemicellulose, cellulose and lignin from the alkaline extraction method

The spider web indicates that there is a difference in water content, yield, hemicellulose content, cellulose content and lignin content resulting from acid extraction method and alkaline extraction method. According to [7], generally the water content is expected to produce a very low water content. This is because cellulose with high water content will cause the ability to save cellulose in short term, thus affecting the utilization of limited cellulose.

According to [12], the higher hemicellulose content and lignin content are decomposed, the higher cellulose content and better cellulose quality will be obtained. The chemical property between the hemicellulose and lignin structures is similar ability of being soluble in alkaline solutions (NaOH). The solution of hemicellulose and lignin leads to the disconnection and breakdown of hemicellulose bonds and lignin into simple sugars and phenolic salts which dissolve easily in water during washing [2].

The alkaline extraction method has higher cellulose content compared to the acid extraction method, because cellulose is soluble in strong acids. The extraction process using a strong acid solution in the acid extraction method transforms cellulose into glucose so that it breaks down and dissolves easily. As a result, the soluble cellulose reduces cellulose content [6].

The yield indicates that acid extraction produces a higher yield than alkaline extraction method. According to [3], the use of NaOH will multiply hemicellulose compounds and degrade lignin in the extraction of tobacco stems. This is in line with [5], who contend that the use of NaOH will damage the components of hemicellulose and lignin which will affect the yield. The resultant yield contains not only cellulose, but also hemicellulose and lignin. The spider web shows thatt alkaline extraction method is the best method because it can separate other compounds such as hemicellulose and lignin on tobacco stems (*Nicotiana tabaccum L.*).

F. FTIR Spectroscopy

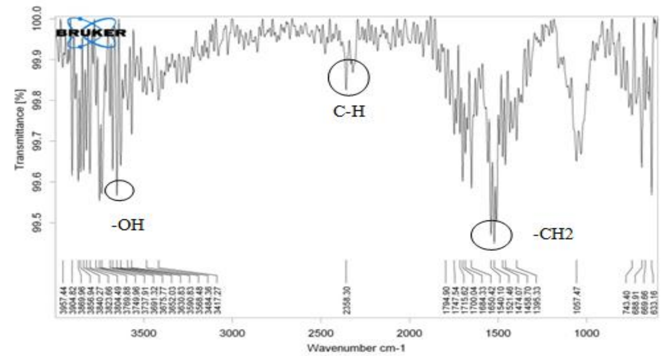

Figure 9. FT-IR Spectrum concentration of 12% alkaline extraction method

According to [10, 4, 15, 20, 7], who have conducted research on cellulose point out that cellulose has important functional groups namely –OH, C-H, and -CH_2. The FTIR spectrum of each treatment shows the existence of cellulose-specific functional groups at wave lengths similar to the strengths of different spectra. FTIR spectrum image shows the presence of a hydroxy group (-OH) vibrational strain with a wave number in the range of 3691.32 cm-1.

The C-H function group is an existing group as a framework for the development of cellulose structure [15, 7] The FTIR spectrum image shows the absorption of the C-H group with a wavelength of 2358.30 cm-1. Wavelengths with a range of 1521.46 cm-1 – 1540.10 cm-1 indicate the presence of an organic compound function group (CH_2) which is a constituent of cellulose structures [10, 7].

IV. CONCLUSION

Acid extraction method and alkaline extraction method based on chemical test results indicate the presence of cellulose content in tobacco stems (*Nicotiana tabaccum L.*). The best extraction method based on chemical tests in producing cellulose content is the alkaline extraction method. Alkaline extraction method results in the water content range of 7.62%-9.66%, the yield range of 43.63%-43.82%, hemicellulose content of 14.33%-16.67%, cellulose content range of 40.33%-46.17%, and lignin content range of 2.83%-6.33%. The FTIR test indicates that there is a functional group that appears on cellulose also found in the FTIR spectrum of each treatment so that the tobacco stems (*Nicotiana tabaccum L.*) has contained cellulose compounds.

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