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ENHANCEMENT OF ANTIMICROBIAL MICRO CELLULOSE OF BAGASSE BY MODIFICATION WITH SILVER NANOPARTICLES

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ABSTRACT

The preparation of microcellulose from sugarcane pulp enzymatically and its modification with silver nanoparticles had been conducted in this study. The objectives of this research were to prepare and characterize microfiber cellulose, and also its application as an antimicrobial material. Micro cellulose has been prepared by enzymatic methods and then continued with ultrasonication. The characterization of microfiber cellulose has been conducted by determining wavelengths with UV-Vis, functional groups with FTIR, surface image with SEM, and crystallinity with XRD. Synthesis of AgNPs had been conducted by bioreduction method using red betel leaf extracts via ultrasonication and stabilized by 0.05% starch solution. Characterization of silver nanoparticles had been conducted by determination of wavelength with UV-Vis and particle size with PSA. The antimicrobial tests of microcellulose have been determined by measuring of clear zones in *Staphylococcus epidermidis, Pseudomonas aeruginosa*, and *Candida albicans.* The characterization of microcellulose showed that wavelengths of micro cellulose at 300 nm, has C-O glycosidic bonds, the C=O, and lignin/hemicellulose. However, C=C lignin aromatic rings begin to disappear. Microcellulose size was 1 μm and crystallinity was 79.05%. The wavelengths in silver nanoparticles were shown at 424 nm. The diameter of silver nanoparticles was 88.6 nm. Micro cellulose with nanoparticle modification was the best in microbial inhibition.

Keywords: Antimicrobials, Enzymatic, Micro cellulose, Red Betel Leaves, Silver Nanoparticles.

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INTRODUCTION

Advanced materials from cellulose with microscales in the current technological developments of the world are very interesting to study.[1] Cellulose is an alternative material because cellulose sources in Indonesia are very abundant and very easy to obtain. The source of cellulose was obtained from bagasse waste which had a cellulose content of 52.42%, lignin of 21.69%, and hemicellulose of 25.8%.[2] The considerable cellulose content in bagasse, bagasse could be a potential material to be used as a source of microcellulose.[3] Micro cellulose from bagasse cellulose was obtained using various methods such as chemical methods, mechanical methods, and biological (enzymatic) methods.[4] Chemical methods are not environmentally friendly because the use of corrosive strong acids can produce hazardous waste[5]. Mechanical methods have the disadvantage of expensive tool production costs and require more energy than chemical methods.[6] Enzymatic methods involving chemical solvents to a minimum allow for this hydrolysis to occur less due to the use of chemicals to minimum compared to acid hydrolysis, enzymatic methods can also increase efficiency and reduce operational costs.[7] Resizing cellulose to micro-sized needs to be combined enzymatic methods and other mechanical methods such as ultrasonication methods.[8] Ultrasonication has the advantage

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of its uncomplicated process making it easy to convert cellulose into micro cellulose at the extraction process and extraction cost, the ultrasonication method also successfully converts cellulose into micro cellulose with a diameter between 2-3 μm.[9] Micro cellulose characterization can use UV-Vis (Ultra Violet – Visible)[10], FTIR (Fourier Transform Infrared), SEM (Scanning Electron Microscopy)[11], and XRD (X-Ray Diffraction).[12] Microbial growth in cellulose is very good due to the inertia that cellulose has and its surface area[13], therefore it is necessary to deposit silver nanoparticles as antimicrobials. Silver nanoparticles are rated to have antimicrobial inhibitory power.[14] The synthesis of silver nanoparticles can use various methods such as physical methods, chemical methods, and biological methods.[15] Physics methods have great energy consumption disadvantages, chemical methods are not environmentally friendly, and expensive, and biological methods have the advantages of being environmentally friendly, and economical.[16] The biology method uses the medium of biological materials such as plants in the synthesis of silver nanoparticles.[17] Red betel leaves contain many substances such as alkaloids, flavonoids, polyphenols, quinones, and saponins as secondary metabolites.[18] Red betel leaf as a bio reductor because it contains phenolic compounds present in flavonoids as a natural reductor because it can reduce Ag^+ to Ag^0.[19] The objectives of this study were to prepare and to characterize microfiber cellulose from sugarcane waste enzymatically, and also to apply this microfiber cellulose after modification by using AgNPs as the antimicrobial material.

EXPERIMENTAL

Material and Methods

The material used is bagasse from PG. Madukismo Bantul, Indonesia, *Staphylococcus epidermidis* ATCC 35984, *Pseudomonas aeruginosa* FNCC 0063*,* and *Candida albicans* SC5314 from the Microbiology Laboratory FMIPA UNY, Indonesia, $AgNO_3$ EMSURE® ACS solids, Reag. Ph Eur., hydrogen peroxide 30%, sodium hydroxide 5%, filter paper, aqueous, *Whatman* no. 41 from Chemix Bantul, and red betel leaf (*Piper crocatum*) from Nologaten, Sleman, Yogyakarta, Indonesia. The instruments were FT-IR *Spectrometer Thermo Scientific Nicolet* iS10, PSA model Microtax FLEX 11.1.06., SEM JSM-6510LA, UV-Vis Shimadzu UV-3600PLUS, 230V, *Ultrasonic cleaner*, Autoclave (Hirayama), *Hot plate* EYELA *magnetic stirrer* RCH-3, sift 200 mesh, glassware, *shaker*, Petri dish, and thermometer.

Preparation of Cellulose

Bagasse was sifted with 200 mesh sifting, as much as 20 grams of bagasse in bleaching with 5% NaOH at 80ºC for 3 hours. Then it was delegated with H_2O_2 30% at a temperature of 80ºC for 1 hour. Next, the fibers are washed to a neutral pH.

Enzymatic Process of Bagasse

A total of 10 grams of cellulose pulp was mixed with 100 mg of xylanase enzyme and hydrolyzed with 50 mL of 0.1 M citrate buffer at 50°C for 48 hours.

Bagasse Ultrasonication Process

A total of 1 gram of fiber in 30 mL of distilled water was ultrasonicated at a temperature of 20°C for 20 minutes.

Synthesis of Silver Nanoparticles

As much as 12.5 grams of red betel leaves were mixture with 200 mL aqueous, the mixture is sorted and heated at 80ºC for 30 minutes then filtered using Whitman 41 filter paper to obtain red betel leaf extract. Then, a solution of 45 mL $AgNO_3$ 0.001 M was mixed with 5 mL of red betel leaf extract. Then, a 50 mL starch solution is added. Then the mixture is ultrasonicated at a temperature of 25ºC for 30 minutes.

Test of Antimicrobial Activity

The *Staphylococcus epidermidis* as a positive bacterium*, Pseudomonas aeruginosa as a* negative bacterium, and *Candida albicans* as a fungus were used in this assay. Antibacterial testing begins with the preparation of NA (Nutrient Agar) and NB (Nutrient Broth) media, while antifungal testing with PDA. NA, NB, and PDA were dissolved in aqua dest with the following ratio: NA: aqua dest = 28 g: 1 L, NB: aqua dest = 13 g: 1 L, PDA: aquadest = 38 g: 1 L. Then boil a solution of NA, NB, and PDA and then autoclaved for 1 hour. Antimicrobial activity testing uses chloramphenicol as a positive control and *paper disc* as a negative

control. Testing of antimicrobial activity in bacteria is carried out once every 3 hours for 48 hours, and testing on fungi is carried out once every 6 hours for 48 hours. The diffusion method in this assay has been conducted by determination of clear zones against the *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Candida albicans.*

Characterization

Microcellulose characterization is carried out by determining wavelengths with UV-Vis, functional groups with FTIR, surface size with SEM, and crystallinity with XRD. The formation of colloidal nanoparticles with reductor of red betel leaves was determined through UV-Vis and PSA. Characterization of silver nanoparticles has been determined by analysis wavelength with UV-Vis and particle size with PSA. Characterization of antimicrobial tests in microcellulose by determination of clear zones against *Staphylococcus epidermidis, Pseudomonas aeruginosa*, and *Candida albicans.*

RESULTS AND DISCUSSION

Bagasse undergoes a *bleaching* process to remove hemicellulose fibers and a delignification process to remove lignin[20], both processes of isolating cellulose from bagasse produce cellulose which is shown in Fig.-1a. Cellulose that has been isolated is subsequently hydrolyzed with enzymes and ultrasonicated to change the cellulose size to a smaller size, namely microfiber cellulose (Fig.-1b).

(a) (b)

Fig.-1: (a) Cellulose (b) Microfiber cellulose

The UV-Vis Analysis of Cellulose and Microfiber Cellulose

Microfiber cellulose has the characteristics of a wavelength of 300 nm (Fig.-2), in agreement with data previously reported in the literature.[10] Microfiber cellulose is more clearly visible than in cellulose, some emerging peaks are possible due to the presence of non-uniformly distributed sizes.

Fig.-2: The UV-Vis Spectrum of Cellulose and Microfiber Cellulose

The FTIR Analysis of Cellulose and Microfiber Cellulose

The functional groups in cellulose and microfiber cellulose are presented in Fig.-3. The representation of the peaks of the cellulose of bagasse is presented in Table-1. Cellulose and microfiber cellulose qualitatively have almost the same functional groups[22], but there have been changes in the functional group's sharpness in microfiber cellulose including C-O glycosidic bonds, C-O glycosidic bond, C=O strain, and the lignin/hemicellulose. However, the C=C lignin aromatic rings begin to disappear in microfiber cellulose.

The SEM Image of Cellulose and Microfiber Cellulose

The surface of cellulose is shown in Fig.-4. It appears that the fibers have not been defibrillating into smaller fibrils, and the uneven distribution of some is in the form of bundles, in agreement with data previously reported in the literature.[23]

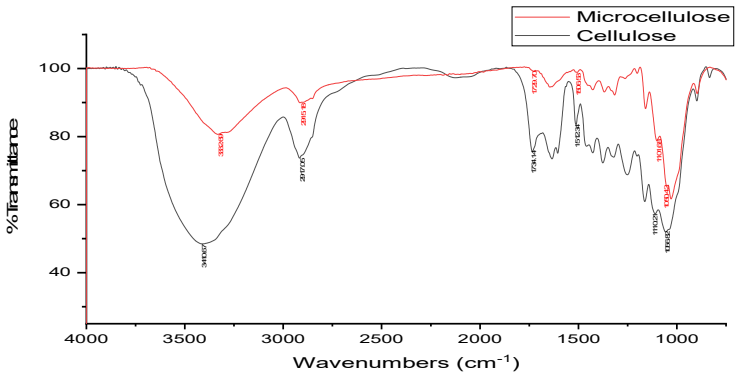

Fig.-3: The Spectrum of FTIR Cellulose and Microcellulose

Table-1: The Functional Groups of Cellulose and Micro cellulose

Functional Groups	Cellulose Wave numbers (cm^{-1})	Microfiber Cellulose Wave numbers (cm^{-1})	Reference
O-H	3410.67	3332.69	11, 9, 21
C-H	2917.05	2915.18	11, 9
Lignin/Hemicellulose	1734.14	1729.07 (Decrease in intensity)	21
Strain C=O	-	1644.68	11
C-O strain	1056.82	1050.42	11
C=C of aromatic ring lignin	1512.34	1506.53 (Decrease in intensity)	21
C-O glycosides	1110.21	1101.68	11

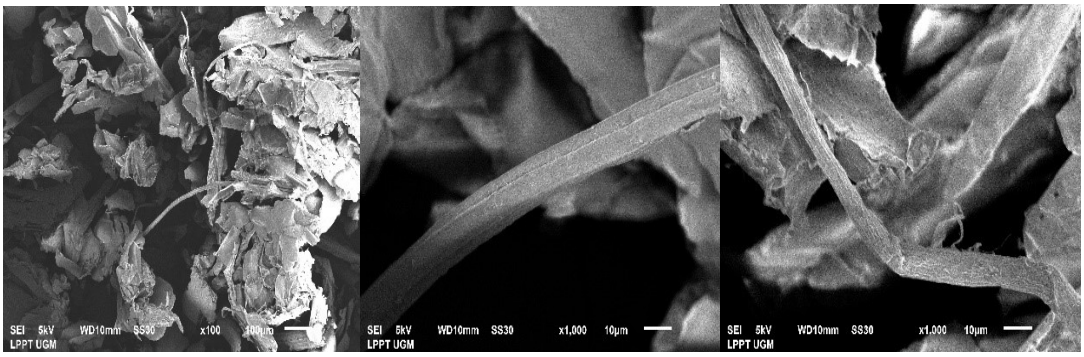

Fig.-4. SEM Images of Bagasse Cellulose

The SEM image of microfiber cellulose of bagasse is shown in Fig.-5. It is seen that the fibril threads begin to break down this is possible because the enzymatic processes carried out have already begun to decompose the lignin and hemicellulose impregnated with the surface of the cellulose.

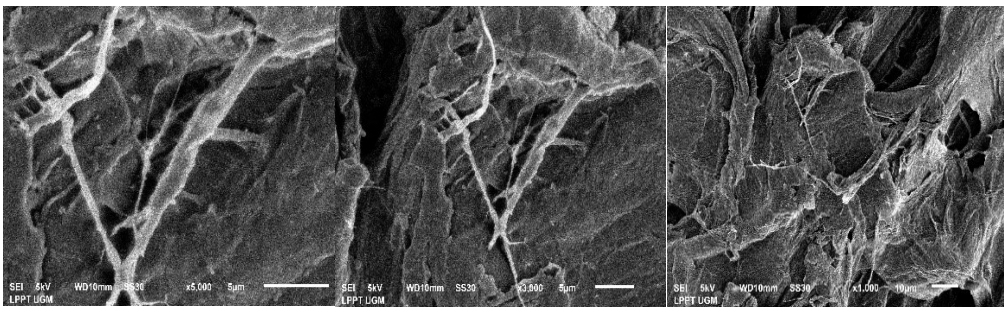

Fig.-5: SEM Images of Microfiber Cellulose

The magnification results at 10,000 times showed a micro cellulose size of 1 μm. The reduction in the size of cellulose into micro cellulose is also characterized by the formation of long stripe shadows, in agreement with data previously reported in the literature.[24]

The XRD Analysis of Cellulose and Microfiber Cellulose

The 2θ of cellulose is 15.16; 20.16; and 25.94 while 2θ in microfiber cellulose is 16.52 and 22.62. The 2θ of cellulose and microfiber cellulose in 15.16^0 shifted to 16.52^0. Then, the loss of 2θ at 20.16^0 and the shift of 2θ at 25.94^0 to 22.62^0.

(a) (b)

Fig.-6: Diffractogram XRD (a) Cellulose and (b) Microfiber Cellulose

The crystallinity index of cellulose is 45.36% and 79.05% for microfiber cellulose. Thus, cellulose is more amorphous than microfiber cellulose. The crystal size of cellulose and microfiber cellulose was calculated by *Scherrer equivalence*, which was obtained at 8.197 nm in cellulose and 1.523 nm in microfiber cellulose. The crystal size data, shows that the crystal size contained in microfiber cellulose is smaller than that of cellulose.

Synthesis and Characterization of Silver Nanoparticles

Figure-7 shows the synthesis of AgNPs, red betel leaves after being stirred and heated at 80ºC for 30 minutes and then filtered using filter paper no. 41 is shown in Fig.-7a. After adding 45 mL of $AgNO_3$ 0.001 M solution and 50 mL of starch solution, the pale pink filtrate is obtained (Fig.-7b) The filtrate was subsequently ultrasonicated (Fig.-7c). The filtrate changes to a darker color that is dark brownish orange (Fig.-7d). This process of discoloration indicates that colloids of silver nanoparticles have already formed. Flavonoids in red betel leaves as a natural reductor can reduce Ag^+ to Ag^0. This led to the formation of colloids of silver nanoparticles.

(a) (b) (c) (d)

Fig.-7: Synthesis of Silver Nanoparticles

The UV-Vis Analysis of Silver Nanoparticles

Characterization of silver nanoparticles was performed in the wavelength range of 200-800 nm as shown in Fig.-8. The stability of silver nanoparticles is formed in the maximum wavelength range of 414-447 nm.[25] The absorbance value is 1.37, this value is influenced by the synthesis time, and the longer the synthesis time the greater the absorbance value.[26]

The Distribution of Particle Size of Silver Nanoparticles

The particle size of silver nanoparticles is shown in Fig.-9.

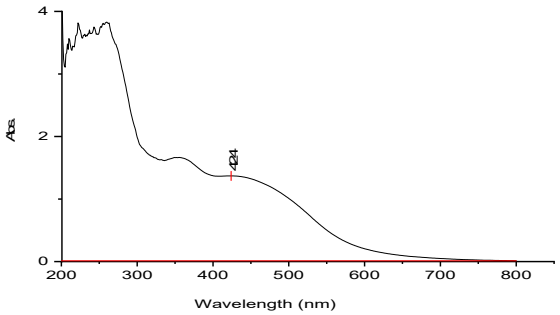

Fig.-8: The Spectrum UV-Vis of Silver Nanoparticles

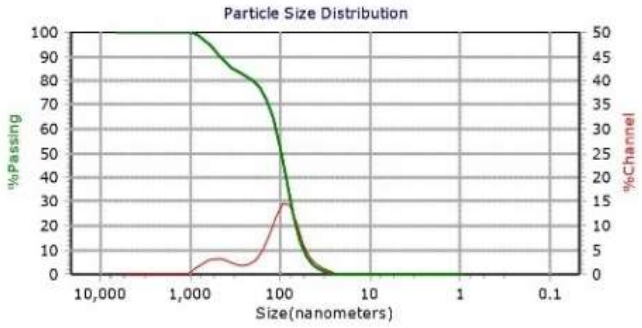

Fig.-9: The Distribution of Silver Nanoparticles (AgNP)

Figure-9 the average particle size diameter of silver nanoparticles with a percentage of 81.9% is 88.6 nm, this is to the previous research.[27] The silver nanoparticles are formed below 100 nm. The remaining percentage of 18.1% indicates that there are still scattered particles with an average of above 100 nm. The article is on average 486 nm in size.[28]

The Antimicrobial Activity Test of Microfiber Cellulose

Tests of microbial inhibition activity on *Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Candida albicans* are shown in Fig.-10.

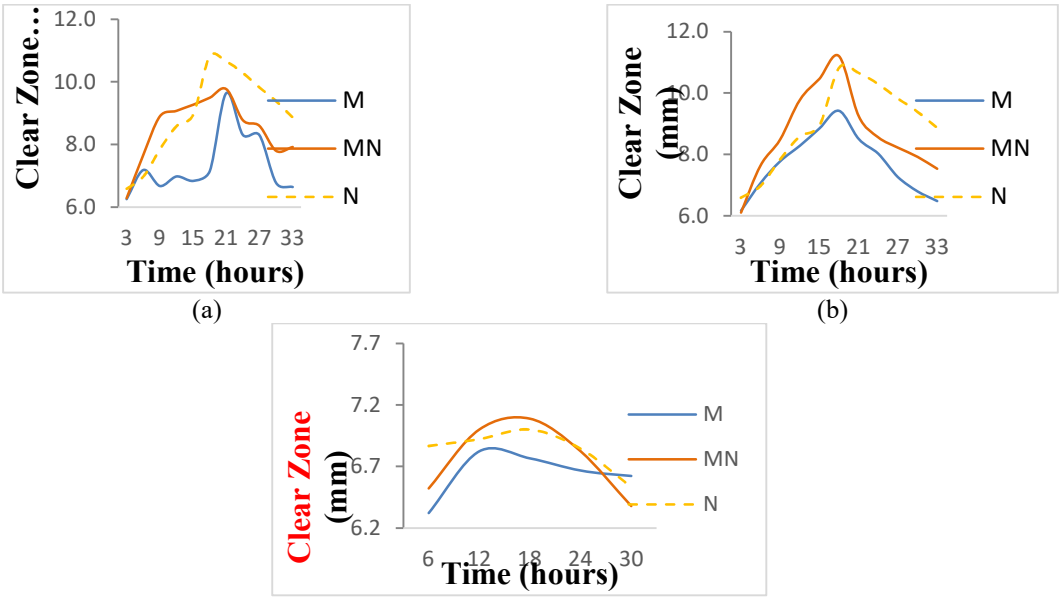

Fig.-10: Clear Zone (a) *Staphylococcus epidermidis,* (b) *Pseudomonas aeruginosa*, (c) *Candida albicans* (The Silver Nanoparticles (N), Silver Nanocellulose Modified Microfiber Cellulose (MN), and Microfiber Cellulose Without Silver Nanoparticles (M))

Microbial inhibition activity based on Fig.-10(a), (b), and (c) shows that the MN has high inhibition compared to M and AgNPs. The AgNPs in a composite of micro cellulose after modification with AgNPs attack the cell membranes in bacteria which contain proteins and sulfur compound as the main component, the Ag compound interacts with the metabolic chain of bacteria and then reacts with DNA until the cells died.[29-31]

CONCLUSION

The microfiber cellulose modified from cellulose has the characteristics of a wavelength of 300 nm, a C-O glycosidic bond, and a C=O strain. The SEM images showed that the size of microfiber cellulose was about 1 μm and based on diffractogram XRD, the crystallinity of micro cellulose was about 79.05%. The AgNPs have a wavelength of 424 nm and a diameter of 88.6 nm. Silver nanoparticles can inhibit microbes. Modification of microfiber cellulose with the addition of AgNPs showed the highest inhibition against *Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Candida albicans*.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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