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Effectiveness of Cellulose Acetate Nanofiber from Banana Leaf Waste (*Musa paradisiaca L.*) as an Antibacterial Filter in Masks

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ABSTRACT

The COVID-19 pandemic requires all people in Indonesia to comply with health protocols, from washing hands to wearing masks. Cloth masks are one of the masks that are widely used by the community. However, the public needs to pay attention to the rules of the cloth masks used. This study aimed to produce an antibacterial filter on masks and to find out the effective and efficient processing of Banana stems as an antibacterial filter on masks. Banana stems were chosen as the raw material because they are easy to obtain, have a cellulose content of ± 63%, and have antibacterial compounds. The stages of this research were sample preparation, phytochemical screening of banana stems, isolation of α-cellulose, and synthesis of cellulose acetate from α-cellulose, which were further characterized by using FTIR, manufacture of dop solution, manufacture of nanofiber membranes by electrospinning, and product application. The resulting product was tested by pressure drop, contact angle, antibacterial activity, SEM, and porosity tests. The test results showed that the resulting product was a nanofiber with a fiber diameter of ± 220.74075 nm that had antibacterial activity, which was indicated by the formation of an inhibition zone with a diameter of ± 14.8 mm in *Escherichia coli* and ± 9.8 mm in *Staphylococcus aureus*, the filter is hydrophilic with an average contact angle of 60°. From the observations, it can be concluded that banana stems have the potential as an antibacterial filter on masks.

Keywords: Antibacterial, Banana Stem, Cellulose, Mask Filter, Nanofiber

ABSTRAK

Pandemi covid-19 mewajibkan seluruh masyarakat di Indonesia untuk mematuhi protokol kesehatan mulai dari mencuci tangan hingga memakai masker. Masker kain merupakan salah satu masker yang banyak digunakan oleh masyarakat. Namun, masyarakat perlu memperhatikan aturan dari masker kain yang digunakan. Tujuan penelitian ini adalah untuk menghasilkan filter antibakteri pada masker serta mengetahui proses pengolahan pelepah pisang yang efektif dan efisien sebagai filter antibakteri pada masker. Pelepah pisang dipilih sebagai bahan baku karena mudah didapat, memiliki kandungan selulosa mencapai ± 63%, dan memiliki senyawa antibakteri di dalamnya. Tahapan penelitian ini adalah preparasi sampel, skrining fitokimia, isolasi α-selulosa, dan sintesis selulosa asetat dari α-selulosa dan dikarakterisasi menggunakan FTIR, pembuatan larutan dop, pembuatan membran nanofiber dengan electrospinning, dan pengaplikasian produk. Produk yang dihasilkan diuji dengan pressure drop, *contact angle*, aktivitas antibakteri, SEM, dan porositas. Hasil pengujian menunjukkan bahwa produk yang dihasilkan berupa nanofiber dengan diameter serat berukuran ± 220,74075 nm, memiliki aktivitas antibakteri yang ditunjukkan dengan terbentuknya zona hambat dengan diameter ± 14,8 mm pada *E. coli* dan ± 9,8 mm pada *S. aureus*, filter bersifat hidrofilik dengan sudut kontak rata-rata 67º. Dari hasil pengamatan, dapat disimpulkan bahwa pelepah pisang memiliki potensi sebagai filter antibakteri pada masker.

Kata Kunci: Antibakteri, Filter Masker, Nanofiber, Pelepah Pisang, Selulosa

1. Introduction

The COVID-19 pandemic requires all Indonesian people to comply with health protocols, from washing their hands to wearing masks. The type of mask needs to be considered for mask use. Cloth masks are one of the masks that are widely used because cloth masks can be made by yourself, can be reused after washing, and can reduce the accumulation of medical mask waste. However, the public needs to pay attention to the rules for cloth masks that can be used. WHO recommends that homemade cloth masks have three layers: the outer layer is made of hydrophobic material, the innermost layer is made of hydrophilic material, and the middle layer has a filtration function and can contain droplets [1].

Medical mask standards that have been drawn up by the French Standardization Association (AFNOR Group), the minimum filtration performance is a minimum of 70% solid particle filtration or droplet filtration and ease of breathing, namely a maximum pressure difference of 0.6 mbar /cm^2. Based on the standard requirements for filtration and breathability, innovation is needed in the form of an antibacterial mask filter suitable for use as a middle layer on cloth masks [2].

The cellulose acetate nanofiber filter is an innovation of a natural filter sheet with tiny pores, which hold large and small splashes to enter. This cellulose acetate nanofiber filter is made from banana stems. So far, banana stems have been regarded as a wasted material rather than being used as a material with high economic value [3]. However, banana leaf waste does not rule out the possibility of being used as a material for making antibacterial filters for masks.

Banana stems were chosen because they are easy to obtain and are environmentally friendly. According to data from the Ministry of Agriculture of the Republic of Indonesia, the harvested area of bananas in Indonesia in 2019 reached around 105,801 Ha. In North Sumatra, it got about 1,814 Ha. In addition, the selection of banana stems is also due to the cellulose inside, which can reach 63% [4]. Banana peels also contain antibacterial compounds derived from alkaloids, phenols, flavonoids, and saponins, thereby increasing the effectiveness of the nanofiber filter [5]. Cellulose acetate has very small pores that can filter the air that will enter the body. This causes only certain particles to penetrate the fabric mask layer [6].

This study aimed to determine the effective and efficient processing of banana stem waste as an antibacterial filter on masks and determine the effectiveness and antibacterial activity of the mask filters produced.

2. Materials and Methods

2.1. Equipment

In this study, the tools used were aluminum foil, scissors, oven, tube reaction, knife, beaker glass, thermometer, hot plate, glass measure, stem stirrer, funnel glass, magnetic stirrers, filters cloth, pH universal, spatula, Whatman filter paper, erlenmeyer, tube reaction, shelf tube reaction, funnel glass, plastic, rubber, sheath hand, dropper, balance analytics, glass watches, statives, and clamps.

2.2. Materials

Materials used in this study were glacial acetic acid (CH_3COOH), hydrochloric acid (HCl), sodium hydroxide (NaOH), nitric acid (HNO_3), sodium nitrite ($NaNO_2$), sulfuric acid (H_2SO_4) sodium hypochlorite (NaOCl), hydrogen peroxide (H_2O_2), acetone, dimethyl acetamide (DMAc), sodium acetate, methanol, and distilled water.

2.3. Sample Preparation

The obtained banana stems were dried in the oven, cut into small pieces, and then crushed with a blender until they became powder.

2.4. Banana Leaf Phytochemical Screening

2.4.1 Alkaloid Test

As much as 2 mL of the extract was evaporated in the beaker glass. The resulting residue was then dissolved with 5 mL of HCl 2M. The solution obtained was divided into 4 test tubes. The Bouchard, Dragendorff, Mayer, and Wagner reagents were added for each tube reaction. In the Dragendorf reagent, an orange precipitate will be formed. The Bouchardat reagent will form a brown precipitate, the Wagner reagent will form a brown precipitate, and the Mayer reagent will include a yellow precipitate, indicating alkaloids.

2.4.2 *Flavonoid Test*

Samples that have been finely dried are put into a beaker glass. Then, ethyl acetate solvent was added until submerged and divided into 4 test tubes. In each tube, reactions were added reagent H_2SO_4, Mg powder, and added HCl 37%, $FeCl_3$ 5%, and NaOH 10%. In 98%, the H_2SO_4 reagent will form a yellowish-orange solution. Meanwhile, in Mg + 37%, HCl reagent will form a pink solution, then in 5% $FeCl_3$ reagent will create a black colloid, and NaOH 10% reagent will form a violet-blue solution, which indicates the presence of flavonoids.

2.4.3 *Saponin and Terpenoid Test*

Samples that had been finely dried were put into a beaker's glass, methanol solvent was added, and the extract was taken and distributed to 2 test tubes, each adding Salkowski reagent and Lieberman, Burchard. In the Salkowski reagent, a brick-red solution will be created, and the Lieberman-Burchard reagent will create a bluish-green solution, indicating the presence of saponins and terpenoids.

2.4.4 *Tannin Test*

Samples that had been finely dried were put into a beaker glass, methanol solvent. Then, the extract was taken and added per e action $FeCl_3$ 1% will produce a change in color of the solution to yellowish green, which indicates the presence of tannins.

2.4.5 *Glycoside Test*

Testing with the Lieberman n-Burchard reaction was carried out through 1 g of banana stem extract entered into a porcelain cup, added with 5 mL of anhydrous acetic acid. H_2SO_4 added 10 drops, a blue or green color occurs, indicating glycosides' presence.

2.5. Isolation of α-Cellulose from Banana Stems

As much as 75 g of banana steam was put into a beaker glass of 5 liters, then 1 liter of a mixture of 3.5% HNO_3 and 10 mg $NaNO_2$ was added and heated on a hotplate while stirring at 90°C for 2 hours, then filtered and washed the residue until the filtrate is neutral. The residue of 1 liter of 2% NaOH solution was heated at 80°C for 4 hours while stirring on the hotplate and filtered back. Then, wash the residue until the filtrate is neutral. The residue obtained was bleached with 1 liter of a solution made from acetate buffer solution and 1.7% NaOCl with a ratio of 1:1 (v/v), heated at 80°C for 6 hours, and stirred on a hotplate, filtered and washed until the filtrate is neutral. The residue obtained was added to 500 mL of 17.5% NaOH solution, heated at 80°C for 30 minutes while stirring on a hotplate, and filtered and washed until the filtrate was neutral. The residue obtained, 500 mL of 10 % H_2O_2 solution, heated at 60°C for 15 minutes while stirring on a hotplate, filtered, and washed until the filtrate is neutral. The residue obtained was dried at 60°C in the oven for 4 hours, stored in a desiccator, and weighed [7]. Alpha cellulose was obtained and then characterized by FTIR (Fourier spectrophotometer Transform Infrared).

2.6. Synthesis of Cellulose Acetate from α-Cellulose

Cellulose synthesis process acetate from α- cellulose using 3 stages: the activation stage, the acylation stage, and the hydrolysis stage. The activation stage was carried out by reacting 2 g of α-cellulose from Banana stems with 50 mL of glacial acetic acid and stirring for 3 hours at a speed of 125 rpm. Stage acetylation by adding 3 drops of concentrated sulfuric acid catalyst and 15 mL acetic anhydride to be stirred again at 25^{o}C with a variation of 3 hours. The solution was then added to 2 mL of distilled water and 5 mL of glacial acetic acid with a reaction time of 30 minutes. The final step is hydrolysis by adding 2 mL of distilled water and 5 mL of glacial acetic acid with a reaction time of 30 minutes. Then, 1 g of sodium acetate into the solution and waited for the process for 5 minutes. A dark-colored solution was formed, then filtered and washed with distilled water until the smell of acetic acid disappeared. Then dried in the oven at 55^{o}C for 6 hours [8]. The cellulose acetate formed was then analyzed for acetyl levels by titration method and characterized using FTIR.

2.7. *Making Dop Solutions*

Preparation of dop solution using solution The precursor in the form of Cellulose Acetate (10 wt % and 15 wt %) was dissolved in Acetone: DMAc (2:1) using a stirrer at room temperature for 2 hours to obtain a homogeneous solution.

2.8 *Manufacturing of Nanofiber Membranes with Electrospinning*

CAAI 2160 Electrospinning tool was switched on to reduce and control humidity (RH). In this experiment, the RH was set at 45%. The solution was put into a syringe (1 mL for the investigation) and a needle (nozzle) with a diameter of 0.6 mm. Then, a high voltage source (HV) was connected to the needle and flow. The syringe pump regulates the solution rate at 1 µL/minute. The preparation is placed on the collector drum, which moves back and forth to distribute the fiber evenly. The distance between the needle tip and the collector is adjusted according to the request. After everything is ready, electrospinning is run. The voltage is adjusted on demand by viewing the cone -jet formed at the tip of the needle through a camera connected to a computer. After obtaining a stable cone -jet, the drum collector is run to collect the formed fiber. In the experiment, the collection was carried out for 3-5 minutes to see the fibers under the microscope. After fiber collection, digital image capture is carried out using a microscope assisted by a camera connected to a computer, and 6 parameters are carried out. After obtaining the best data results from the 5 parameters, it was repeated 2x for further testing.

2.8 *Product Testing*

2.8.1 Pressure Drop Test

The membrane/filter to be tested for pressure drop is placed in the filter holder. Then, the dry air is pulled by the pump and filtered using a HEPA filter to get dry and clean air. The pump controls the airflow rate entering the filter holder (QIN), and the value is confirmed by the flowmeter so that an airflow rate of 0-5 LPM is obtained. Then, the pressure difference before and after the membrane/filter is measured by a differential pressure sensor at each air flow rate.

2.8.2 Test contact Angles

The tested samples were placed above the sample holder that can arranged and located by the sample motor. Then, to observe and take an image, a contact angle was used to connect the camera _ to the GUI on the computer and given the source light (backlight). Next, water as the test liquid is adjusted to have a volume of 5 µL before being laid on a sample. After the water on the needle has the desired volume, the syringe is moved lower until the water hits the surface sample, and then the syringe is pushed up so that the water droplets are on top of the surface sample. Next, take a digital image for 10 seconds. For example, change the mark contact angle every time. After taking the image, measure the contact angle using the computer's GUI.

2.8.3 Antibacterial Activity Test

Use a sterile tube containing reaction suspension bacteria. Then, it is smeared on nutrient agar media. Then, nanofiber paper discs from cellulose acetate were placed on top of the media, and smeared bacteria with paper discs A little pressed to stick to the surface of the media. Furthermore, it incubated at 37°C for 24 hours. Activity antibacterial stated positively if an inhibition zone is formed as a clear zone around the paper disc.

2.8.4 SEM Test (Scanning Electrons Microscopy)

The electron waves emitted by the electron gun condense in the condenser lens and are focused as a clear point by the objective lens. Scanning the energized coil provides a magnetic field for the electron beam. The electron beam striking the sample generates secondary electrons, which are then collected by the secondary or backscatter detector. The resulting image consists of thousands of points of various intensities on the surface of the Cathode Ray Tube (CRT) as the topography of the image. In this system, the electron beam is concentrated on the specimen, and the image is magnified with an objective lens and projected onto a screen.

2.8.5 Porosity Test

A porosity test was carried out to determine the amount of substance or component that can be absorbed by the membrane. A porosity test was carried out on isopropyl alcohol so you can know the amount of isopropyl alcohol that can be absorbed by nanofiber. The porosity test is done by immersing the membrane in isopropyl alcohol for 24 hours at room temperature, and then the nanofiber is weighed. Afterward, the nanofibers were dried in a vacuum oven at 60°C for 48 hours until completely dry and weighed.

2.8.6 Porosity Test

Mask filters will enter the cloth mask to help slow down the deployment of bacteria.

3. Results and Discussion

3.1 *Screening Phytochemicals Banana Stems*

Phytochemical screening was conducted to test the compounds in the sample by making extracts from banana stems that have been finely dried and then tested using specific reagents. The results showed that Banana stems contain alkaloid compounds, phenols, flavonoids, and saponins with antibacterial properties. The flavonoid screening results can be seen in Table 1.

Table 1. Flavonoid Screening Results of Sample Banana Stem

Compound Metabolites Secondary	Reactor	Results
Alkaloids	*Bouchardat*	+
	Mayer	+
	Dragendorff	+
	Wagner	+
Steroids and Terpenoids	*Salkowski*	-
	Liebermann- Burchard	-
Saponins	Aquadest+Alcohol 96%	+
Flavonoids	$FeCl_3$ 5%	+
	$Mg_{(s)} + HCl_{(p)}$	+
	NaOH 10%	-
	$H_2SO_{4(p)}$	-
Tannins	$FeCl_3$ 1%	-
Glycosides	*Liebermann- Burchard*	-

Description :
(-) : No detected Compound Metabolites Second
(+) : Detected Compound Metabolites Secondary

3.2. *Isolation of α-Cellulose from Banana Stems*

The total yield obtained was 22.0338 g of colored α-cellulose white. The FTIR spectra results from banana stem isolation show cluster function found in α-cellulose. In the wavenumber region of 3265.1 cm^{-1} indicates the vibrational peak of the -OH group, at wave number 2892.4 cm^{-1} indicates the stretching vibrational of CHS, at 1371.7 cm^{-1} indicates the vibrational peak of $-CH_2$ bending, at wave number of 1021.3 cm^{-1} shows the vibrational peak of the COC group which is the bond between C1-C4 and the β-1,4-glycosidic bond.

3.3. Synthesis Cellulose Acetate of α- Cellulose

Total results obtained as much as 10 g of cellulose acetate colored white. The FTIR spectrum results from the synthesis of cellulose acetate show groups function in cellulose acetate. The region of wave number 3287.5 cm-1 offers the vibrational peak of the -OH group. In wave number 2892.4 $cm^{-1,}$ it shows the vibrational peak of stretching CH. In wave number 1722.0 $cm^{-1,}$ it is characteristic of the carbonyl group C=O ester, and wave number 1021.3 cm^{-1} shows the characteristics of the cluster CO. This shows that qualitatively cellulose acetate has undergone esterification so that cellulose acetate products have been formed. Acetyle content from cellulose the acetate obtained of 48.19%.

3.4. Pressure Drop Test Results

This test evaluates the pressure drop before and after air passes through the membrane. Using a test area of 3.14 cm 2. The pump controls the incoming air flow rate, and the flowmeter confirms the value so that an airflow rate of 0–5 LPM (Liters per minute) is obtained. Table 2 shows a pressure drop from 3 experiments ranging from 55.79 Lm^{-1}. The pressure drop increases with increasing air flow rate through the membrane.

Table 2. Pressure Drop Sample

Sample	Pressure Drop @ 5.3 cm/s
Measurement 1	55.71 ± 0.05
Measurement 2	55.81 ± 0.05
Measurement 3	55.87 ± 0.05

3.5. Contact Angle Results

Contact angle testing was done to show a material's hydrophilic or hydrophobic properties (hydrophobicity). The contact angle is defined as the angle formed by two lines, where the first line is the boundary between the air and the dripped liquid, and the second line is the boundary between the liquid and the dripped solid. When a liquid is dropped on a solid in open air, the liquid will be in an equilibrium state a few moments after the drop. A contact angle is formed in this state, as shown in Figure 1.

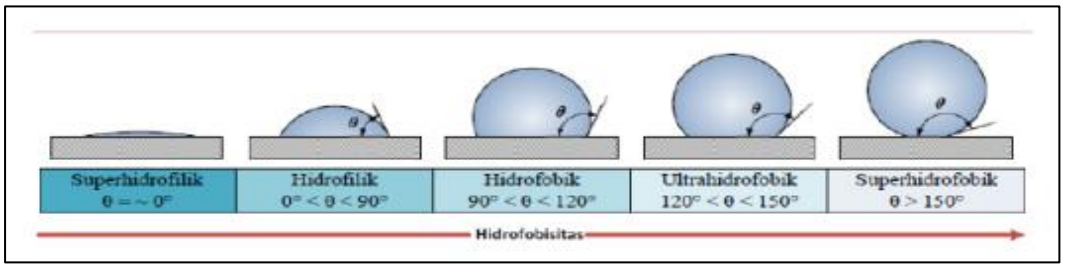

Figure 1. Water contact angle image [9]

The roughness of a surface influences wettability, and wettability on the surface can be measured by the contact angle of the water above the surface of the material. A surface is hydrophilic when the contact angle is $0^{\circ} < \theta < 90^{\circ}$ and hydrophobic when the contact angle is $90^{\circ} < \theta < 120^{\circ}$.

For nanofiber filter material, the test was carried out with variations in time starting from 0 to 18 minutes at a temperature of 29.1 $^{\circ}$C. Water as the test liquid was adjusted to have a volume of 5 µL before being placed on top of the sample. The capture is carried out every 10 seconds to see changes in contact values angle every time. The results show that the nanofiber filter has an average contact angle of 67° at 0 minutes and continues to decrease to 21° at -18 minutes. These results indicate that the cellulose acetate nanofiber filter is hydrophilic, as shown in Table 3.

Table 3. The contact angle test results

No.	Time (minute)	θ_{left}	θ_{right}	$\theta_{is\ average}$	Category
1	0	64.776 ± 0.035	69.655 ± 0.041	67.215 ± 0.076	Hydrophilic
2	2	61.02 0 ± 0.062	62.798 ± 0.079	61.909 ± 0.141	Hydrophilic
3	4	56.71 0 ± 0.035	63.593 ± 0.47 0	60.152 ± 0.081	Hydrophilic
4	6	52.728 ± 0.046	59.648 ± 0.065	56.188 ± 0.111	Hydrophilic
5	8	47.689 ± 0.44 0	55.593 ± 0.07 0	51.641 ± 0.114	Hydrophilic
6	10	43.094 ± 0.58 0	50.84 0 ± 0.091	46.967 ± 0.15 0	Hydrophilic
7	12	36.902 ± 0.55 0	46.054 ± 0.128	41.478 ± 0.183	Hydrophilic
8	14	34.487 ± 0.136	36.27 0 ± 0.147	35.378 ± 0.283	Hydrophilic
9	18	21.974 ± 0.299	21,902 ± 0.222	21.938 ± 0.521	Hydrophilic

3.6. Activity Test Results Antibacterial

According to Davis and Stout (1971), the criteria for the strength of antibacterial power are as follows: namely, the diameter of the inhibition zone ≥ 20 mm, the response is very strong, 10-20 mm the response is strong, the diameter of the inhibition zone is 5-10 mm the response is moderate, and the diameter of the inhibition zone is ≤ 5mm weak response [10]. The bacteria used in this test are *Escherichia coli* and *Staphylococcus aureus*. These two microorganisms were selected based on the research results [11]. In this study, the bacteria successfully isolated from masks were *E.coli* (54%) and *S.aureus* (25%). The results of the antibacterial test on cellulose acetate nanofibers can be seen in Table 3.

Table 3. The results of the antibacterial test on cellulose acetate nanofibers

Bacteria	Sample	Inhibition Zone Diameter (mm)	Strength Power antibacterial
Escherichia coli	N1	6	Currently
	N2	32.2	Very Strong
	N3	26.1	Very Strong
	N4	14.8	Strong
Staphylococcus aureus	N1	6	Currently
	N2	27.4	Very Strong
	N3	7.4	Currently
	N4	9.8	Currently

Description :
N1 : Nanofiber dissolved acetone
N2 : Nanofiber is dripped tetracycline
N3 : Nanofiber dripped amoxicillin
N4 : Nanofiber without treatment

3.7. SEM (Scanning Electron Microscopy) Analysis Results

The membrane nanofiber's structure morphology can be determined using the instrument SEM (Scanning Electron Microscopy). The results of this SEM test aim to know the structure obtained on the surface. The nanofiber was carried out at magnifications of 2000x, 5000x, 10,000x, and 15,000x to show uniformity size scattered pores evenly, tightly, and homogeneously. Nanofiber with 10,000x magnification shows uniform size pores in a clear, even, tight, and homogeneous manner so that nanofiber cellulose acetate can be used for antibacterial mask filters. The size obtained by the nanofiber is 220.74075 nm.

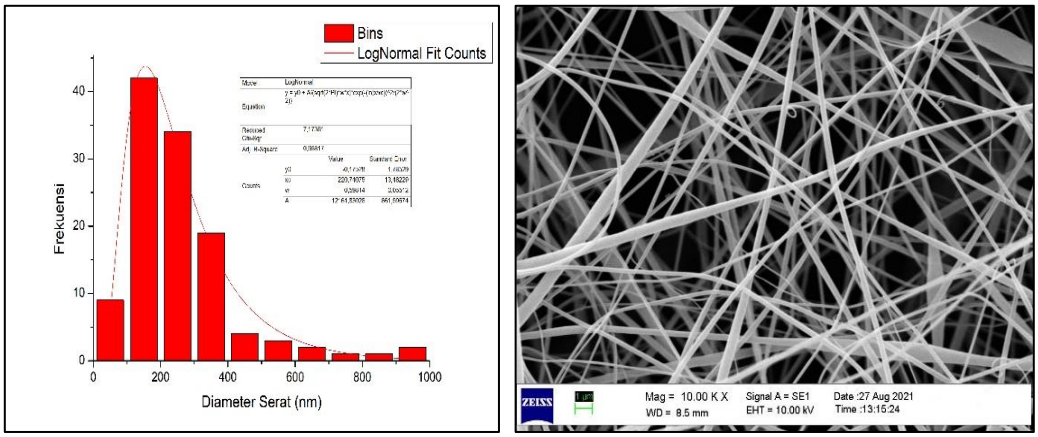

Figure 2. The SEM image of cellulose acetate

3.8. Porosity Test Results

Testing porosity was done to influence temperature coagulation to pore the resulting membrane. Porosity is one possible factor that affects the performance of the membrane. Its tiny resulting pores will impact the performance membrane in determining the flux. Based on the results of this study, it got 35.96%.

3.9. Application Product

The obtained mask filter will be measured following the available filter size entered into the cloth mask, then the inserted filter mask on the layer in the middle of the cloth mask. The antibacterial filter on the mask is ready to be used.

4. Conclusion

The method used in the manufacture of nanofiber cellulose acetate from waste banana stems (*Musa paradisiaca* L.) as The antibacterial filter on the mask is sample preparation, screening of banana midrib

phytochemicals, isolation of α-cellulose from banana stems, synthesis of cellulose acetate from α-cellulose, preparation of dop solutions, manufacture of nanofiber membranes by electrospinning, product testing includes pressure testing. The drop test, contact test angle, antibacterial activity test, SEM analysis, porosity test, and product application. The resulting mask filter is effective because it has a fiber diameter of 220.74075 nm and antibacterial activity, as indicated by an inhibition zone diameter of ± 14.8 mm in *Escherichia coli* and ± 9.8 mm in *Staphylococcus aureus*.

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6. Conflict of Interest

Authors declare no conflicts of interest

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