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EFFECT OF NANO CHITOSAN ON THE CHARACTERISTICS OF WET WIPE FROM COCOA HELL WASTE

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ABSTRACT

Cocoa husk is one of the agricultural wastes that can be utilized as raw material for pulp making, because cocoa contains cellulose so that it can be used as raw material for tissue making. Natrium hydroxide is used to isolate the cellulose in cocoa. Nano chitosan was prepared by varied chitosan mass then reacting with sodium tripolyphosphate with ionic gelation for the formation of nanostructures and freezing methods to reduce water and the formation of gel structures. The purpose of this study was to find the effect of nano chitosan concentration on tissue characteristics from cocoa skin waste, to find the effect of nano chitosan concentration on the effectiveness of anti-bacterial pathogen and to find the optimum chitosan mass. The results obtained on the effect of chitosan mass on the characteristics of wipes from cocoa skin waste are that it can absorb about 20% of the 300 cm2 area and for the appearance of the wipes it is not easy to fade for the color of the wipes themselves and is less clean, not soft and not hollow, testing of pathogen bacteria according to SNI 8526: 2018 obtained the effect of chitosan weight on pathogen bacteria obtained the result that nano chitosan is effective in killing Pseudomonas aeruginosa bacteria in various variations of chitosan weight and optimum chitosan weight at a weights of 4 grams.

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Keywords: Anti-bacterial, Cocoa Shell, Nano chitosan, Wipes, Pseudomonas aeruginosa

1. Introduction

Indonesia is the world's third-largest cocoa producer, with cocoa production reaching 657,050 tons in 2017. The most commonly utilized part of the cocoa plant is its beans, while cocoa fruit husks account for the largest waste byproduct of cocoa production, comprising 75 percent of the total waste (Pratyaksa, Ganda Putra, and Suhendra, 2020).

Cocoa fruit consists of 74% fruit husk, 2% placenta, and 24% beans (Erlita, 2016). Cocoa fruit husks contain chemical components such as lignin, polyphenols, and theobromine. These cocoa fruit husks are currently only utilized as fertilizer and animal feed, but they still have limitations due to their high lignin content. Therefore, the high lignin content in cocoa husks needs to be removed to utilize them in tissue production (Pratyaksa, Ganda Putra, and Suhendra, 2020).

Tissue is one of the forest extractive products. Like paper, tissue is made from paper pulp made from wood fibers. According to a survey conducted by WWF-Indonesia in collaboration with the creative agency Hakuhudo, as reported on http://ditjenppi.menlhk.go.id, Indonesian urban residents tend to use three tissue sheets to dry their hands. Globally, WWF estimates that approximately 270,000 trees are felled and end up in landfills every day. And 10% of that number comes from toilet tissue. One can imagine how many trees need to be felled daily to meet human tissue needs (admin, 2018).

Due to this, in order to reduce deforestation for tissue production, alternative materials such as cassava peel, coconut fiber, and banana peel

waste are considered. However, the author prefers cocoa husks as the raw material for tissue production due to the abundance of cocoa husk waste in the author's environment and to pursue innovative research. Therefore, research on tissue production from cocoa fruit husks is necessary. The cellulose content in cocoa husks can be utilized for tissue production. Thus, the high lignin content in cocoa husks needs to be removed for tissue production (Pratyaksa, Ganda Putra, and Suhendra, 2020). Lignin can be separated through delignification methods (Darojati Purwadi, and Rasrendra, 2020). Delignification is a preliminary process for removing lignin from lignocellulosic materials.

Antibacterials are substances that can disrupt or even kill bacteria by interfering with the metabolism of harmful microbes. Antibacterials include antibiotics and chemotherapy. Antibiotics are substances produced by microbes, especially fungi, that can inhibit the growth or eradicate other types of microbes. Antibiotics can also be synthesized. According to BPOM at https://pionas.pom.go.id/ioni/bab-5-infeksi/51-antibakteri, the classification of antibiotics includes Penicillin, Cephalosporins and other beta-lactam antibiotics, Tetracyclines, Aminoglycosides, Macrolides, Quinolones, Sulfonamides and trimethoprim, and other antibiotics.

Another natural biomaterial with antibacterial activity used as an alternative treatment is chitosan, extracted from the shells of marine animals such as crabs and shrimp. Chitosan is a bioactive material with many advantages, especially in drug delivery. Many experts currently employ nanotechnology to process chitosan and produce substances called nano chitosan.

Nano chitosan has better absorbency and antibacterial and antifungal properties than chitosan alone. Moreover, nano chitosan is non-toxic, stable during usage, has a high surface area, and can be used as a carrier for various types of drugs and plant extractions (Khairunnisa et al., 2020).

Chitosan is one of the compounds that can be utilized in the pharmaceutical field as an antibacterial agent. Chitosan is a derivative compound of chitin with the chemical formula poly (2-amino-2-deoxy-β-D-glucose) (Rizki et al., 2014). Utilizing chitosan as an antibacterial agent is due to its positive charge that can interact with the negatively charged bacterial cell surfaces, thus inhibiting bacterial colony growth. Chitosan is highly potential as an antibacterial agent because it is a natural polymer derived from chitin compounds, thus expected to be safe for humans (Rizki et al., 2014).

Wet wipes that use alcohol as an antibacterial agent are considered less safe for health because alcohol is an organic solvent. Another substance found in hygiene products is methylisothiazolinone (MIT). Several studies indicate that MIT can cause allergic reactions and exhibit cytotoxicity (Rizki et al., 2014). Personal care products also often contain triclosan as an antibacterial agent. While triclosan is reported to be non-toxic, some cases of dermatitis or skin irritation have been found after exposure to triclosan. This indicates that triclosan may be photoallergic and cause contact dermatitis (PACD). Based on this, it is necessary to find other safe and effective antibacterial agents (Rizki et al., 2014).

The application of chitosan as an antibacterial agent in wet wipes is considered safe, thus further development is needed. The demand for increasingly practical lifestyles is also one of the reasons for conducting research aimed at developing wet tissue products that are safe for users. The resulting wet tissue products

are diverse, besides being hand sanitizer substitutes, they can be developed into wet wipes specifically for toddlers (baby wipes), napkins, and bottle wipes. Chitosan wet wipes are wet tissue products that use chitosan as an antibacterial agent to prevent and inhibit bacterial growth (Rizki et al., 2014). Nano chitosan is more reactive against bacterial cells than chitosan alone, as it is easily absorbed into bacterial cells faster. However, the determination of nano chitosan concentration as a hand sanitizer is still unknown (Hardiningtyas, Bahri, and Suptijah, 2022). Therefore, this research will produce wet wipes from cocoa husk waste with the addition of environmentally friendly and skin-safe nano chitosan antibacterial agents.

2. Material and Methods

Materials for Nano chitosan preparation

Distilled water, 2% Acetic Acid, CETA (Cetrimide Agar), Chitosan, MCA (Mac Conkey Agar), MCB (MacConkey broth), MSA (Mannitol Salt Agar), RVSEB (Rappaport Vassiliadis Salmonella Enrichment Broth), STPP (Sodium tripolyphosphate), TSA (Tryptic Soy Agar), XLDA (Xylose Lysine Deoxycholate Agar)

Materials for Tissue preparation

Distilled water, 2% Hydrogen Peroxide (H2O2), Chitosan, Cocoa Fruit Skin, Tapioca Flour, VCO (Virgin Coconut Oil)

Tools

Sieve, Balp Filler, blender, Mold, Hot Plate, Magnetic Stirrer, Oven, Centrifuge, Vortex

Research Methodology

Tissue Preparation

Twelve grams of raw materials and NaOH solution are added to a beaker at a ratio of 1:50. The beaker is then covered with aluminum foil. Put it in the oven at 80°C for 45 minutes. The cooked result is filtered to separate it from the cooking solution (black liquor). Print on a square-shaped mold then bake at 105°C. Put the pulp sheet in a beaker and add 500 ml of H_2O_2. Cook the solution for 1 hour at 60°C. Filter and rinse with distilled water. Add 0.3 g of tapioca flour, and 4 ml of VCO. The pulp is then printed on a 50 mesh fiber mold with an area of 20 x 30 cm.

Nano Chitosan Preparation

Chitosan is weighed with variables of 0.5 g, 1 g, 1.5 g, 2 g, 2.5 g, and 4 g dissolved in 2% acetic acid 300 ml stirred with a magnetic stirrer. 0.5% STPP is added as much as 100 ml and stirred for 100 minutes until an emulsion is formed. To reduce the water content, the solution is placed in the freezer for 24 hours. (the emulsion solution will freeze, then melt, and the water will be removed). The emulsion solution is centrifuged at a speed of 10,000 rpm for 30 minutes. The resulting chitosan nano emulsion is stored in a bottle and placed in the refrigerator for freezing. Characterized by XRD and PSA.

Application of Nano Chitosan in Wet Tissue

Tissue made from cocoa fruit skin is placed at the bottom of the container and nano chitosan solution is slowly added. The tissue is ready for use.

Analysis Method

Tissue Characteristic Analysis

Tissue appearance Prepare each tissue sample and observe the tissue by filtering and transillumination. Tensile test Insert tissue into water then let it stand and observe. Absorption test Prepare a paper strip with a width of 15 mm and a length of 200 mm, Hang the tissue perpendicular to the water surface after 10 minutes, read the height of the absorbed water.

Antibacterial analysis

Prepare each bacterium with a concentration of 10^4 and take 1 mL of each

bacterium then mix using a vortex. Mix 0.1 mL of bacteria with each variable mass of nano chitosan then homogenize using a sterile stirrer. Swab the bacterial suspension mixed with nano chitosan and insert it into TSB media then vortex. Pipette 2 mL from the TSB tube then place it in each petri dish as much as 1 ml. Pour TSA media then homogenize, let it stand until solidified, incubation at 32°C for 5 days, after 5 days observe the results.

Biochemical analysis

Take the colonies found in the previous test then streak on TSA media Incubate at 32°C, take bacteria using a round ose then place it in MCB, RVSEB, CETA, MSA media incubate at 32°C for RVSEB, CETA, and MSA while for MCB incubate at 42°C for 18-24 hours. For MCB and RVSEB take using a round ose then continue to MCA and XLD media then Incubate again at 32°C for 18-24 hours. Observe the results of each media.

3. Results and Discussion

Research Results Data obtained from the testing of Tissue Characteristics are presented in Tabel 1, 2 and 3 as follows:

Table 1. Tissue Appearance Results

NaOH Concentration	Results
0,1 N	Less clean, not soft, not porous
1 N	Less clean, not soft, not porous
1,5 N	Less clean, not soft, not porous
2 N	Less clean, not soft, not porous

Table 2. Wrinkle Test Results

NaOH Concentration	Result
0,1 N	Non fading
1 N	Non fading
1,5 N	Non fading
2 N	Non fading

Table 3. Absorbency Test Results

NaOH Concentration	Result mm
0,1 N	32
1 N	35
1,5 N	30
2 N	41

Discussion In this study, after conducting characteristic tests on the tissue, it was found that the optimum solvent concentration is with 2N NaOH solvent. The author used 2N NaOH solvent to delignify cellulose for better separation of cellulose from other substances during tissue making. For the concentration of 2N NaOH solvent, the results obtained were less clean, not soft, and not perforated in the tissue appearance test, not fading in the wrinkle test, and for the absorbency test, an absorbency of 41mm out of 200mm was obtained, equivalent to 20% of the tissue area. In this test, a comparison was made with the previous study entitled "Cassava Peel (Manihot Utilissima) as an Alternative Material for Tissue Paper Making" by Paula Tyasmita Andar Ningrum.

The update in this research is the use of raw material from brown skin. Although the results obtained are not as good as tissue made from cassava peel, the reference journal for the next study by the author is "Optimization of Tissue Making from Banana Stem Kepok Using Organosolv Method with Microwave Heating" by Widi Aprilia Ta'bi N. the update in this research is the non-use of ethanol solvent in the delignification process, but instead using NaOH solvent. This is because when the author attempted to make tissue from both solvents, it was found that NaOH solvent could produce better tissue than ethanol solvent.

In the production of nano chitosan, it was found that during testing with XRD, the

chitosan formed was amorphous, while in PSA testing, the average particle size obtained was 1416.9 nm, with the standard size of nano particles being 0.1-12,300 nm. This research is compared to a previous study titled "The Effect of Chitosan Mass Variation on Yield in the Production of Chitosan Nano emulsion using Ionic Gelation and Freezing Methods" conducted by Sari, et al (2020). The previous study only investigated the effect of chitosan mass variation on yield in the production of nano chitosan, while in this study, an update was made to determine the effect of chitosan mass variation as nanochitosan on pathogenic bacteria, which had not been done in previous research.

Figure 1. PSA Graph of Chitosan Nanoemulsion

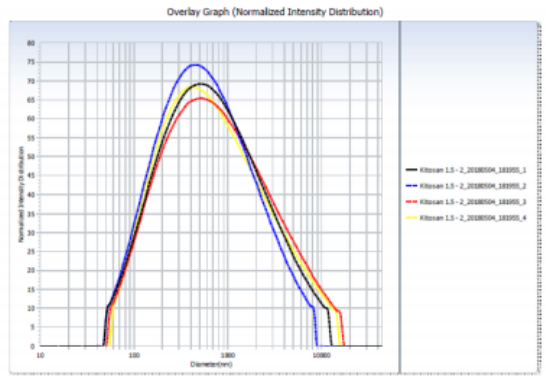


Figure 1. XRD Graph of Chitosan Nanoemulsion

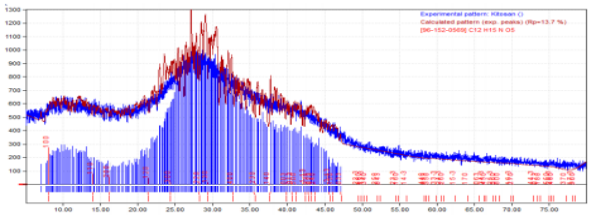


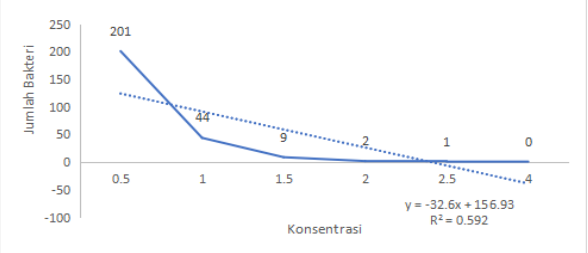
Table 4 Results of TPC Testing

Chitosan mass (Gram)	Total Plate Count (Cfu/Plate) Result					
	1	2	3	4	5	Rate
0,5	21	90	147	193	211	201
	35	87	131	175	190	
1	3	17	29	40	45	44
	9	23	35	42	42	
1,5	0	1	3	5	6	9
	0	2	5	9	11	
2	0	1	2	3	3	2
	0	0	0	0	0	
2,5	0	1	1	1	1	1
	0	1	1	1	1	
4	0	0	0	0	0	0
	0	0	0	0	0	
Alcohol 70 %	0	0	0	0	0	0
	0	0	0	0	0	
Positif	59	105	128	156	173	177
	69	101	137	162	181	
Negatif	0	0	0	0	0	0
	0	0	0	0	0	

Table 5. Results of Biochemical Testing

Chitosan mass (Gram)	Bakteri			
	E. Coli	S. aureus	P. aeroginosa	Salmonella T
0,5	+	+	-	+
1	+	+	-	+
1,5	+	+	-	+
2	+	+	-	+
2,5	+	+	-	-
4	-	-	-	-
Alcohol 70 %	-	-	-	-
Positif	+	+	+	+
Negatif	-	-	-	-

Figure 2. Linearization of the Effect of Nanochitosan on Pathogenic Bacteria



From the graph of the effect of nano chitosan on pathogenic bacteria, the author obtained results indicating that the higher the concentration of nano chitosan used, the greater its ability to kill pathogenic bacteria. This can also be seen from Table 9, where the concentration tested at 4 grams was able to kill all standard microbiological test strains. According to the SNI 8526:2018 standard, concentrations ranging from 1 gram to 2.5 grams for Total Plate Count (TPC) testing meet the requirements. However, in biochemical testing, they did not meet the requirements because they were only able to kill Pseudomonas bacteria. Nevertheless, when using 4 grams, the results met the requirements according to the SNI 8526:2018 standard, as they were able to kill the test bacteria, and in TPC testing, no bacteria grew.

The researcher compared these findings with a previous study titled "Comparison of the effectiveness of antiseptic wet tissue products in inhibiting bacterial growth using the replica method" by Sadheli & Riniwasih (2021). This research brought innovation by using a different antibacterial substance, nano chitosan, and adding variations of pathogenic bacteria tested, aiming to determine which bacteria could survive and which could not.

Conclusion

From this research, it can be concluded that: there is no influence of chitosan mass on tissue characteristics in terms of tissue appearance and color fastness, while it is derived from cocoa skin waste. However, there is an influence of chitosan mass on pathogenic bacteria, namely in all concentration variations used can inhibit bacterial growth, but only the weighing variation with a weight of 4 grams can kill all pathogenic bacteria (E. coli, Salmonella.T, S. Aureus, and Pseudomonas A). An optimum chitosan mass was obtained that can be used as an antibacterial substance for wet tissues in accordance with SNI 8526:2018, namely with a weight of 4 grams of chitosan.

Suggestions

Further testing is needed to strengthen the possibility that nano chitosan can be used as an antibacterial agent with other methods or to find a more optimal concentration within the weight range of 2.5 grams to 4 grams. If nano chitosan is used as an antibacterial agent on tissues, additional substances must be used to mask the smell of acetic acid and for the color and texture of the tissues themselves, which still do not resemble tissues made from wood.

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