Synthesis and Characterization of Chitosan-Based Hand Sanitizer From Crab Shell Waste

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Abstract— Washing hands with soap and running water for 20 seconds is the most effective way to prevent the development of bacteria, germs, and viruses, including the Covid-19 virus. However, with such a busy life, time for washing hands becomes a problem. Hand sanitizer comes as a solution to this problem. However, some hand sanitizer gels on the market still use alcohol. The use of alcohol in hand sanitizer is considered less safe for health because alcohol can dissolve the fat layer on the skin which functions as a protection against microorganism infection. On repeated use it can cause dryness and irritation of the skin. One of the natural ingredients that can replace the use of alcohol in hand sanitzers is chitosan. The purpose of this study was to produce a hand sanitizer made from chitosan from crab shells produced by 2 different producers. The hand sanitizer was made by mixing chitosan in a 2% acetic acid (CH3COOH) solution added with a solution of carboxymethyl cellulose (CMC) using a magnetic stirrer at 5000rpm for 90 minutes. Bacterial inhibition test was carried out on the resulting hand sanitizer and alcohol-based commercial hand sanitizer as a comparison. The results showed that the commercial hand sanitizer had the best bacterial inhibitory ability, with a value of 82.5 mm. Meanwhile, the chitosan-based hand sanitizer in sample 2 produced the inhibitory ability of bacteria which was close to the commercial hand sanitizer with a value of 81.25 mm.

Index Terms— Covid-19, hand sanitizer, alcohol, chitosan, CH3COOH, carboxymethyl cellulose.

1 Introduction

Amidst the Covid-19 virus pandemic, Indonesian government is campaigning for a hand washing movement to prevent the spread of the Covid-19 virus. Washing hands with soap and running water for 20 seconds is the most effective way to prevent the development of bacteria, germs, and viruses, including Covid-19. However, with such a busy life, for washing hands becomes a problem. When in public places or on public transportation, it can be difficult to find soap and water to keep your hands clean and healthy. Hand sanitizer comes as an effective and efficient product as an alternative to keep hands clean and healthy. Since the Covid-19 virus pandemic, hand sanitizers have become rare and expensive due to massive purchases by Indonesians [1]. As an example, the e-commerce company Tokopedia which sold 72 thousand units of hand sanitizers in 42 minutes during the Covid-19 pandemic [2]. In addition, Sirclo e-commerce recorded a 585% increase in sales of hand sanitizers under the Antis brand during March 2020 [3].

However, several types of hand sanitizer gels on the market still use alcohol as an antibacterial agent. The use of alcohol in hand sanitizer is considered less safe for health because alcohol is an organic solvent that can dissolve the layer of fat and sebum on the skin which functions as a protection against microorganisms infection. In addition, alcohol is flammable, and on repeated use it causes dryness and irritation of the skin [4], [5]. The search for alternative hand sanitizer formulations that are safe for health has been widely carried out in line with the increasing negative impact on health, as well as the increasing desire of people to use natural ingredients or "back to nature". This is responded by the number of products made from natural ingredients that are used for health care. One of the natural ingredients that can replace the use of alcohol in hand sanitzers is chitosan [6]

Chitosan is one of the compounds that can be used in the pharmaceutical field as an antibacterial [6], [7]. Chitosan is also called a natural biocompatible polymer because it comes from natural ingredients. By that, its use does not cause side effects, is non-toxic, and biodegradable [8]. The main source of chitin and chitosan is the shells of Crustaceae sp, namely shrimp, lobster, crab, shellfish, crabs and other shelled animals, especially those from the sea [9] [10]. Chitosan can be used in antibacterial activity because it has a polycationic nature which can inhibit the growth of bacteria and fungi, has a high killing rate against bacteria, and low toxicity toward mammalian cells [11]. The research study by Paomephan et. al., investigated the influence of molecular weight (Mw) and particle size characteristics of chitosan nanoparticles (CNs) on the antibacterial property for application a a vegetable wash disinfectant. The wash solution containing CNs was found to be the most effective in killing more than a 1 log reduction of both inoculated E. coli and S. Typhimurium populations, The results of this study indicate that CNs can be used as an effective disinfectant for washing fresh vegetables [12]. Dragostin et. al., using chitosan derivatives as a new antimicrobial for wound dressing applications. The new chitosan derivatives can used as a new material of dressing for wounds, especially for burn wounds because the derivatives of chitosan showed improved swelling and biodegradation rate, biocompatible, non cytotoxic and thus improved healing effects [13].

Therefore, this study uses chitosan from small crab shells produced by two different producers. The objectives of this research are to produce hand sanitizer made from chitosan synthesized from crab shell waste.

2 METHODS AND MATERIALS

The manufacture of hand sanitizer in this study used chitosan made from crab shells produced by the CBIOM3s Lab (sample 1) and Chimultiguna (sample 2). 0.5gr carboxymethyl cellulose (CMC) was dissolved in 10 ml of aquadest using a magnetic stirrer at a speed of 100 rpm at 60°C for 10 minutes to produce a carboxymethyl cellulose solution as shown in Figure 1.

Figure 1. Manufacturing Process of Carboxymethyl Cellulose (CMC) Solution

Figure 2 shows the preparation of the initial solution for hand sanitizer by dissolving 0.75g of chitosan in 100 ml of 2% acetic acid (CH3COOH), then stirring it using a magnetic stirrer at a speed of 5000 rpm for 1 hour. After 1 hour, carboxymethyl cellulose solution was added then stirred until homogeneous with a magnetic stirrer at a speed of 5000 rpm for 90 minutes as shown in Figure 3.

Figure 2. Manufacturing Process of the Hand Sanitizer Initial Solution

Figure 3. Mixing Process of Carboxymethyl Cellulose (CMC) Solution into the Hand Sanitizer Initial Solution

After obtaining a hand sanitizer as shown in Figure 4, the final step is to test the hand sanitizer inhibition against Escherechia coli bacteria. at the Laboratory of Biology, Universitas Negeri Semarang, Indonesia. Meanwhile, as a comparison, testing the inhibition of Escherechia coli bacteria was also performed on commercial alcohol-based hand sanitisers (sample 3).

Figure 4. Chitosan-based Hand Sanitizer

3 RESULTS AND DISCUSSION

The bacterial inhibition test of Escherechia coli bacteria in sample 1, sample 2 and sample 3 were carried out with 2 repetitions as shown in Figure 5. The results of the bacterial inhibition test are shown in the table 1. Commercial hand sanitizer (sample 3) has the best bacterial inhibitory ability. This is indicated by the largest average diameter value of bacterial inhibition, 82.5 mm. While sample 2 has the lowest diameter of bacterial inhibition. In this study, sample 3 produced a diameter of bacterial inhibition that was close to or almost the same as the diameter of bacterial inhibition produced by a commercial hand sanitizer (sample 3).

Table 1. Result of Bacterial Inhibition Tests of the Hand Sanitizers

Sample	Measurement of Bacterial Inhibition Diameter	Diameter of Bacterial Inhibition (mm)	Average Diameter of Bacterial Inhibition (mm)
1	1	80	81.25
	2	82.5	
2	1	65	67.5
	2	70	
3	1	80	82.5
	2	85	

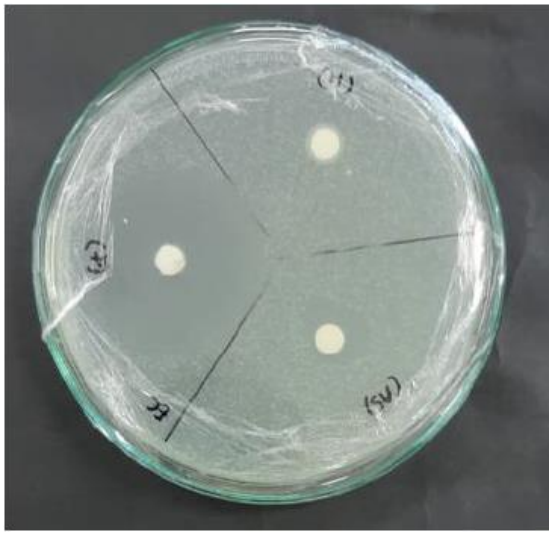

Figure 5. Nano Antibacterial Test VS. +: positive control (Amoxylin); AS: commercial hand sanitizer; H: hand sanitizer made from chitosan

Sample 1 had the same bacterial inhibitory properties as the commercial hand sanitizer (sample 3). This happened because in sample 1, the hand sanitizer was made from chitosan made from crab shell produced by CBIOM3s Lab with the addition of a depolymerization process using hydrogen peroxide (H_2O_2) with a concentration of 13% for 4 hours at 40°C. Temperature regulation during the depolymerization process accelerated the depolymerization process of the chitosan main chain so that the resulting chitosan molecular weight decreased. The lower the molecular weight of chitosan, the higher the solubility rate [14] [15]. So that the depolymerization of chitosan allows chitosan to dissolve in water and has a high antibacterial ability which can be used as an antiseptic for hand sanitizer [15]. The use of chitosan with a smaller size was very effective in inhibiting E. coli bacteria. While the growth of S. Typhimurium bacteria can be inhibited effectively using larger chitosan [13].

Another factor that causes the bacterial inhibition in sample 1 to be better than sample 2 is the degree of deacetylation in chitosan which is used to make a hand sanitizer. The degree of deacetylation (DD) is an important parameter used to define biopolymers whether chitin or chitosan (≥ 40%). This parameter also affects on physicochemical, mechanical properties, and biological properties such as biocompatibility and biodegradability [16]. In this study, the degree of deacetylation was calculated using Formula [17] :

$$DD = 100 - [\left(\frac{A_{1655}}{A_{3450}}\right) x \frac{100}{1.33}] \quad (1)$$

Where A_{1655} and A_{3430} is the absorbance at 1655 cm^{-1} and 3430 cm^{-1} obtained from the results of the FTIR test for each sample shown in Figures 6 and 7.

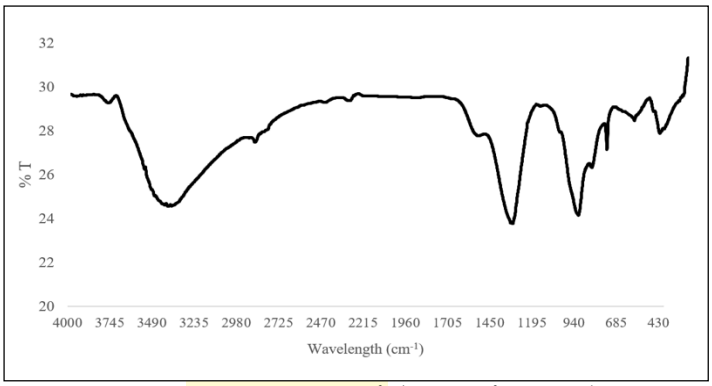

Figure 6. FTIR spectrum of chitosan for sample 1

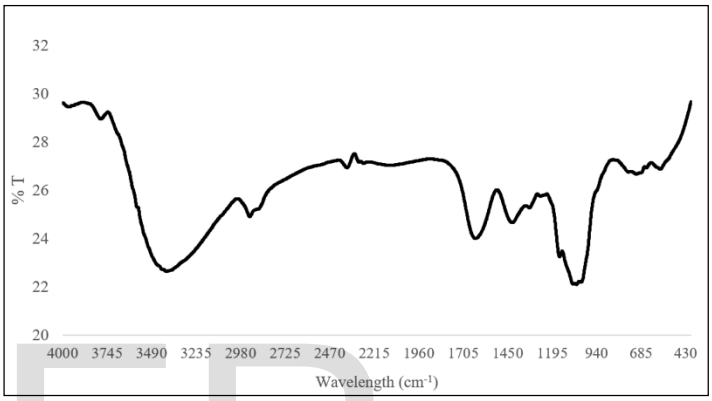

Figure 7. FTIR spectrum of chitosan for sample 2

Sample 1 used chitosan with a deacitilization degree of 91%, whilst sample 2 used chitosan with a deacitilization degree of 52%. The relationship between the degree of deacytilization of chitosan and the inhibition of hand sanitizer bacteria is shown in Figure 8. The higher the degree of deacytilization in chitosan which is used to make hand sanitizer, the higher the bacterial inhibition [18]. The research study by [18] and [19] also found the use of chitosan with a high degree of deacitilization to be very effective in inhibiting bacteria growth.

Figure 8. Relationship Between the Degree of Deacetylation of Chitosan and the Bacterial Inhibition of the Hand Sanitizer.

4 CONCLUSIONS

The use of chitosan as a substitute for alcohol in the manufacture of hand sanitizers has been thoroughly investigated. Commercial hand sanitizer (sample 3) made from alcohol produced the best inhibitory power on Escherechia coli bacteria, while the hand sanitizer made from chitosan, the best bacterial inhibition was found in sample 1 with an inhibitory value close to commercial hand sanitizers. The higher the deacitilization degree of the chitosan used to make hand sanitizers, the higher the bacterial inhibitory ability.

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