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Synthesis and Antimicrobial Activity of Silver N-Methyl Chitosan

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ABSTRACT. This study synthesized silver N-methyl chitosan (Ag-NMC) and tested it for its antimicrobial and antifungal activity. Ag-NMC was characterized by FTIR, XRD, measured for its molecular weight (MW), solubility, and toxicity. The antimicrobial activity was tested by the agar diffusion method, determining the MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration) against *Staphylococcus aureus* and *Escherichia coli* bacteria, and determining the Minimum Fungicidal Concentration (MFC) against the fungus *Candida albicans*. The results showed that Ag-NMC had MW, solubility, and LC₅₀ of 555.65 g/mol, 50 mg/mL, 945,492 mg/L, respectively. The diameter of the inhibition zone from the resulting diffusion test showed that Ag-NMC had better antimicrobial activity than N-methyl chitosan (NMC) and chitosan. The MIC, MBC, and MFC values of Ag-NMC were always lower than that of NMC and chitosan.

Keywords: Antimicrobial activity, Antifungal activity, Chitosan, Silver N-methyl chitosan.

INTRODUCTION

Chitosan is a biopolymer derived from N-acetylglucosamine and glucosamine (Paula et al., 2020) which are obtained from the deacetylation of chitin (Kurniasih et al., 2018). Chitosan can be used in the food, medicine, and biotechnology industries. This is because chitosan is non-toxic, biocompatible, biodegradable, and provides activities of antibacterial (Sudatta et al., 2020), antioxidant (Abd El-Hack et al., 2020), and antifungal (Kurniasih et al., 2018). The antibacterial and antifungal activity of chitosan is influenced by the value of the degree of deacetylation (DD) and molecular weight (MW) (Wang et al., 2020). Low solubility in aqueous media is an obstacle in the application of chitosan (Kulkarni et al., 2017). Chitosan which has a molecular weight of 100 - 200 kDa has poor solubility in water (Wei et al., 2019).

Modification of the C2-amine, C3-hydroxyl, and C6-hydroxyl groups through chemical reactions can produce chitosan derivatives (Paula et al., 2020). Chemical modification can produce chitosan compounds which are more soluble in water (Kurniasih et al., 2018), (Abd El-Hack et al., 2020). Some chitosan derivatives have higher water solubility than chitosan, such as carboxymethyl chitosan (Abd El-Hack et al., 2020) and N-methyl chitosan (NMC). NMC has antifungal activity against *Candida albicans* (*C. albicans*) (Kurniasih et al., 2018) and antioxidant

activity (Kurniasih et al., 2018). Modification of chitosan to NMC will produce amphiphilic chitosan derivatives because they have both hydrophilic and hydrophobic chains (Bobu et al., 2011).

Silver nanoparticles (Ag-NPs) are metal nanoparticles that have been explored for their potential in various applications, such as antimicrobial agents (Gopinath et al., 2020). It has been reported that Ag-chitosan exerts antibacterial activity and antifungal activity (Kalaivani et al., 2018). Ag-NPs with a large surface area can adhere to cell membranes, easily forming free radicals that penetrate cell membranes and separate membrane lipids that cause cell death (Siddiqi et al., 2018). One of the efforts to increase the antibacterial activity of chitosan can be done by synthesizing Ag-NPs based on chitosan. In this study, Ag-NMC was synthesized and then physicochemical characterization was carried out and antimicrobial activity was tested. The microbes used were gram-negative bacteria, gram-positive bacteria, and fungus.

EXPERIMENTAL SECTION

Material

The materials used in this study were chitosan (DD = 88.57%), NaOH (Merck), NaCl (Merck), CH₃COOH (Merck), CH₂O (Merck), NaBH₄ (Merck), AgNO₃ (Merck), BaCl₂·2H₂O (Merck), and H₂SO₄

(Merck), tween 80 (Merck), Luria Bertani Agar (LBA) (Culgenex), Luria Bertani Broth (LBB) (Himedia), Sabouraud Dextrose Broth (SDB) (Criterion), Sabouraud Dextrose Agar (SDA) (Merck), shrimp larvae *A. salina*, bacteria *E. coli* ATCC 35218 and *S. aureus* ATCC 25923 from the Microbiology Laboratory of the Faculty of Medicine Ull Yogyakarta, and the fungus *C. albicans* InaCC Y1574 Center for Biotechnology Research LIPI. The instruments used in this study were FTIR spectrophotometer (Shimadzu 8201), UV-Vis spectrophotometer (A & E Lab's), and XRD (Rigaku Miniflex 600).

Synthesis of N-methyl Chitosan and Silver N-methyl Chitosan

N-methyl chitosan (NMC) was synthesized by modifying the previously reported method (Kurniasih et al., 2018). In a 50-mL volumetric flask, we prepared a 1%(w/v) chitosan solution by dissolving 0.5 g of chitosan in 1% acetic acid. A total of 1.7 mL of 10% formaldehyde was added and stirred for 1 hour. 1 M NaOH solution was added until the pH of the solution was 4.5. A total of 2.6 mL of 10% NaBH₄ (w/v) was added and stirred for 1.5 hours. The result was precipitated by conditioning the pH to 10 using 1 M NaOH. The precipitate was filtered and washed with distilled water. The NMC precipitate was then dried.

The synthesis of silver N-methyl chitosan (Ag-NMC) was carried out by modifying a previous study (Akmaz et al., 2013). NMC 0.2% (w/v) solution was prepared by dissolving 0.1 g of NMC in 0.25% acetic acid in a 50 mL volumetric flask. The solution was added 2 mL of 0.06 M AgNO₃ and 0.1 mL of 0.3 M NaOH (the pH of the solution was adjusted to 10.4). The color of the solution would turn yellow and there was a reddish-yellow precipitate after the addition of the NaOH solution. The solution was stirred constantly for 10 minutes at 95 °C. After 10 minutes the solution was filtered and neutralized with distilled water. The Ag-NMC precipitate obtained was then dried.

Characterization of NMC and Ag-NMC

NMC and Ag-NMC were characterized by FTIR spectrophotometer. Based on the IR spectra than the value of the degree of deacetylation (DD) (Khan et al., 2002) and the degree of substitution (DS) NMC (Bobu et al., 2011) were calculated according to equations 1 and 2. Determination of DD was based on the absorption ratio at 1655 cm⁻¹ from the amide group and 3450 cm⁻¹ from the hydroxyl group. NMC and AgNMC were also identified by XRD (X-ray Diffractometer), molecular weight (Kurniasih et al., 2018), solubility in 1% acetic acid (Kurniasih et al., 2018), and toxicity (Kurniasihurwat et al., 2018) were calculated.

$$DD = 100 - \left[\left(\frac{A_{1655}}{A_{3450}} \right) \times 115 \right] \quad (1)$$

$$DS = \frac{(DD \text{ chitosan}) - (DD \text{ NMC})}{100} \quad (2)$$

Antimicrobial Activity Test Against *E. coli* Bacteria, *S. aureus* Bacteria, and *C. albicans* Fungi Agar Diffusion Method

Cultures of 100 µL of microorganisms (10⁵ CFU/mL bacteria and 10⁶ CFU/mL fungi) were spread on LBA and SDA media, respectively. Then a 10 mm well was made in the middle of the petri dish and added 50 µL of sample solution with a concentration of 400 ppm. The petri dish was then incubated at 37 °C for 24 hours. Antimicrobial activity was calculated by measuring the zone of inhibition by equation 3.

$$\text{Inhibition zone diameter} = \frac{\text{Clear zone diameter} - \text{Well diameter}}{3} \quad (3)$$

Minimum Inhibitory Concentration (MIC) Test

A total of 2 mL of bacteria (10⁵ CFU/mL) and fungi (10⁶ CFU/mL) were added to a test tube containing 2 mL of LBB and SDB media, respectively. The mixture was homogenized by vortex for 1 minute. After that, 2 mL of samples with various concentrations (5, 10, 50, 100, and 200 ppm) were added to different test tubes and homogenized. The test tubes were incubated at 37 °C for 24 hours. The incubation results were diluted with 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴ dilutions. A total of 100 µL of the 10⁻², 10⁻³, and 10⁻⁴ dilutions were put into a petri dish containing 15 mL of solid media (LBA for bacteria and SDA for fungi), then leveled with drugalsky. Petri dishes were incubated at 37 °C for 24 hours. Evaluation of antimicrobial activity was carried out by counting the number of bacteria using the Total Plate Count (TPC) method and the optical density (OD) value by spectrophotometry.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) Tests

The preset MIC values were used to define the range of MBC and MFC values. The least sample concentration without visible growth was considered the minimum killing concentration of microorganisms (*E. coli* bacteria, *S. aureus* bacteria, and *C. albicans* fungi).

RESULTS AND DISCUSSION

Synthesizing N-methyl Chitosan (NMC) and Silver N-methyl Chitosan (Ag-NMC)

Chitosan was produced through the process of chitin deacetylation. The deacetylation process would produce amines that gave polycationic properties to chitosan (Sudatta et al., 2020). The used chitosan had a degree of deacetylation (DD) of 88.57% with a solubility value of 26.19 mg/mL. For the application of chitosan to be wider, it was necessary to increase the solubility of chitosan. Increasing the solubility could be undertaken by making chitosan derivatives, including N-methyl chitosan (NMC) (Kurniasih et al., 2018). NMC could increase solubility at neutral pH values, increase antioxidant activity (Kurniasih et al., 2018), and anticandida activity (Kurniasih et al., 2018).

NMCs were synthesized via reductive amination. The primary amino group in chitosan underwent a Schiff reaction with an aldehyde to form an imine. After being reduced by sodium borohydride it became NMC. The percentage of yield obtained from the synthesis was 74.19%. The reaction for the formation of NMC can be described in **Figure 1**.

The interaction between NMC and Ag metal through the formation of chelates is shown in **Figure 2**. The -NH_2 , -NHCH_3 , and -OH groups would donate lone pairs of electrons to the empty orbitals of Ag. The formation of Ag-NMC was indicated by the formation of a reddish yellow precipitate. The yield of Ag-NMC obtained was 87.86%.

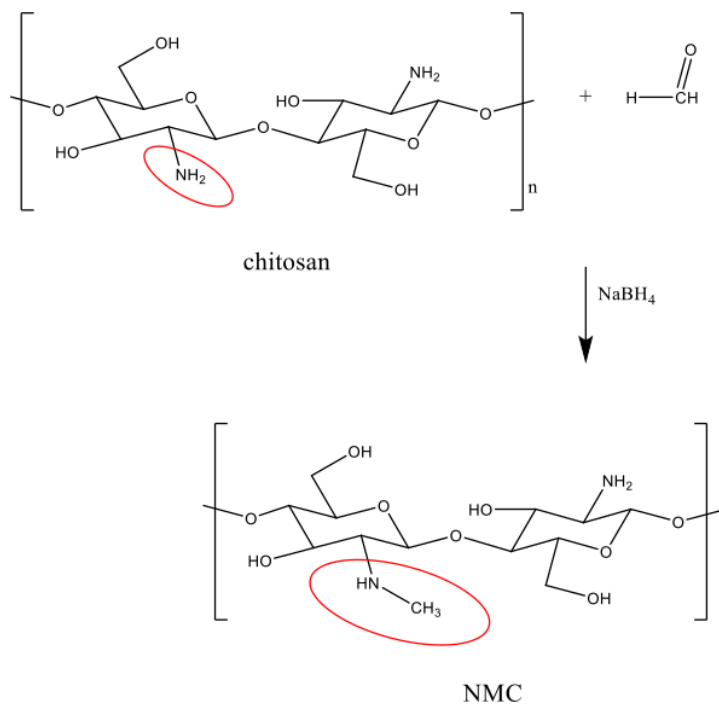


Figure 1. The reaction for the formation of NMC

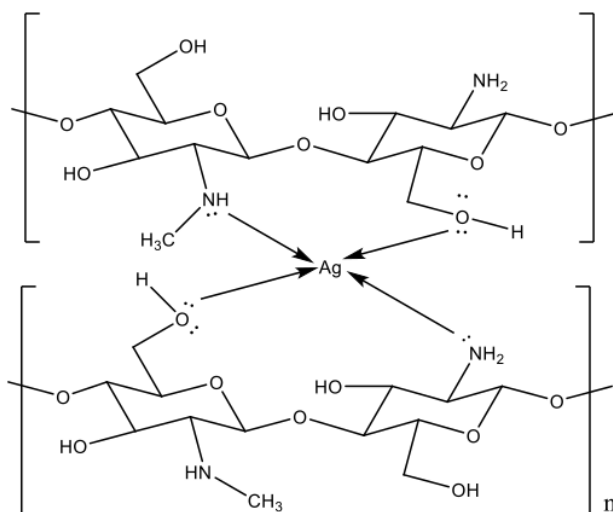


Figure 2. The interaction between NMC and Ag

Physicochemical Properties

The IR spectra in **Figure 3** were used to confirm the specific peak and calculate deacetylation degrees (DD) values of NMC. The peak at 3425 cm^{-1} shows the stretching vibrations of the $-\text{OH}$ (Gopinath et al., 2020) and $-\text{NH}$ groups which overlap each other (Rahimi et al., 2019). The weak peak at 2924 cm^{-1} indicates $-\text{CH}$ stretching at $-\text{CH}_2-$ aliphatic. The characteristic peak at 1597 cm^{-1} indicates that the $-\text{NH}_2$ group from the chitosan has been converted to $-\text{NH}$ with one alkyl substituent. The wavenumber of 1427 cm^{-1} shows the asymmetric $\text{C}-\text{H}$ bond of the NMC methyl group. The interaction between Ag metal and the $-\text{NH}$ group causes a shift in the $\text{C}-\text{H}$ peak to 1419 cm^{-1} .

The synthesized chitosan and NMC had DD values of 88.57% and 85.10%, respectively. Based on equation 2, the DS value of NMC was 0.0347. This meant that 3.47% of the total NH_2 in chitosan had been substituted by methyl. The DS value could be increased by adding aldehyde to the chitosan when the reaction reached equilibrium.

Analysis of the crystallinity of Ag-NMC was carried out using XRD. The results of the XRD analysis are presented in **Figure 4**. The NMC pattern shows the main peaks at 2θ around 20° and 10° which are characteristic of chitosan. The Ag-NMC pattern shows 2θ peaks at around 20° , 10° , and 38° . A peak of 2θ at 38° indicates the peak of Ag according to the Joint Committee in Powder Diffraction Standards (JCPDS) (No. 89-3722). The 2θ peak at 38° is the peak of the Ag (111) crystal phase (Akmaz et al., 2013).

NMC is a polymer with low crystallinity. The hydrogen bonds that occur between the $-\text{OH}$ and $-\text{NH}_2$ groups of NMC intramolecularly and intermolecularly can be illustrated in **Figure 5**. Ag causes damage to the intramolecular and intermolecular hydrogen bonds of NMC by forming chelates between Ag and NMC. The decrease in the intensity of the NMC peak is due to the decrease in the crystallinity of the NMC after the presence of Ag metal in the NMC. An increase in the number of metal ions bound to chitosan results in a decrease in the crystallinity of chitosan (Modrzejewska & Dorabalska, 2009).

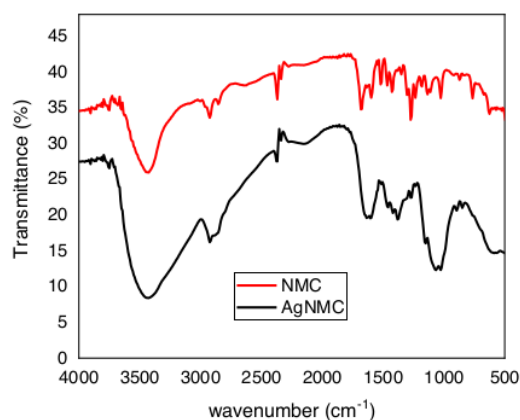


Figure 3. The IR spectra of chitosan, NMC dan Ag-NMC

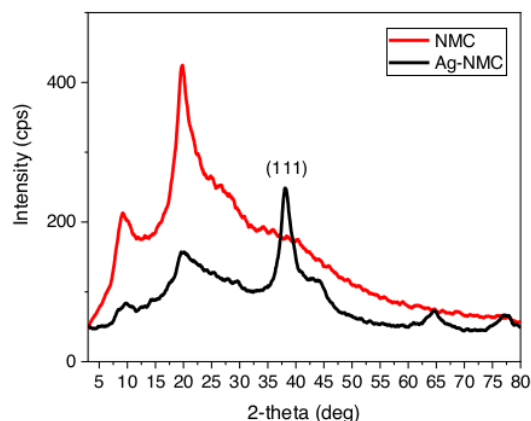


Figure 4. XRD patterns of NMC dan Ag-NMC

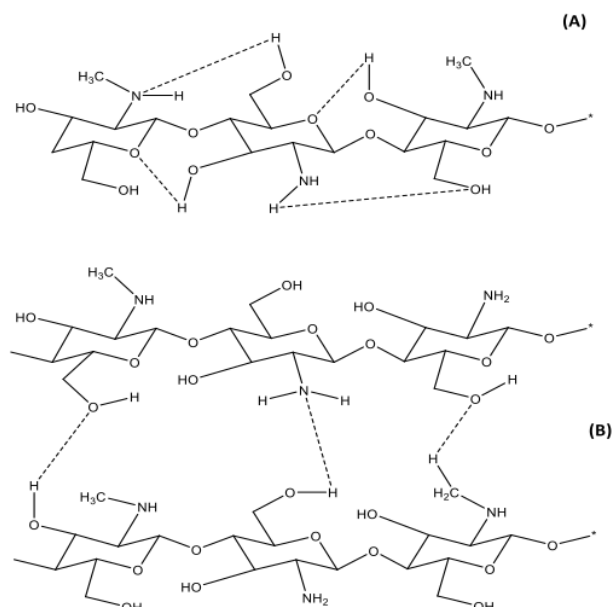


Figure 5. Intramolecularly hydrogen bonds (A) and intermolecular hydrogen bonds (B) of NMC

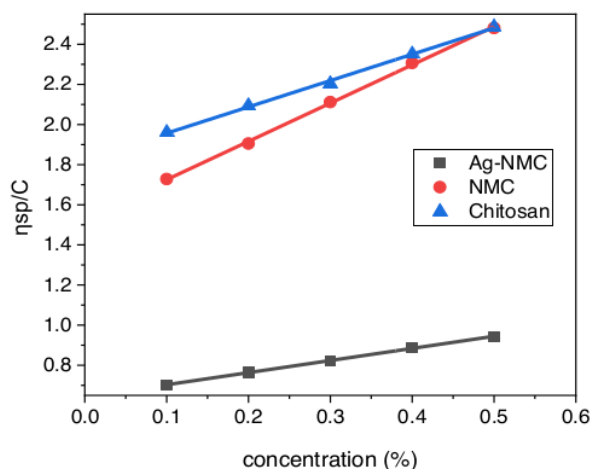


Figure 6. Relationship between concentration and reducing viscosity

Molecular weight (MW) of NMC and Ag-NMC samples were measured based on intrinsic viscosity (η). The data obtained is graphed η_{sp}/C against C as shown in Figure 6. MW is determined based on the Mark-Houwink equation, $\log [\eta] = \log K_m + \alpha \log M_w$, where K_m is the solvent constant (1.81×10^{-3}) and constant α is 0.93. The calculation results show that the MW of chitosan, NMC, and Ag-NMC are 1707.4 g/mol, 1815.72 g/mol, and 555.65 g/mol, respectively. The smaller the MW of chitosan, the easier it can be applied as an antibacterial agent.

The solubility of synthesized Ag-NMC and NMC was 50 mg/mL and 40 mg/mL, respectively. This value was higher than the solubility value of chitosan, which

was 26.19 mg/mL so that NMC and Ag-NMC had greater potential to be applied in various fields. NMC is a chitosan derivative that has amphiphilic properties (Bobu et al., 2011). The amphiphilic nature can dissolve compounds in polar or non-polar solvents because they have hydrophilic and hydrophobic groups. The hydrophilic group possessed by Ag-NMC as an antibacterial makes it soluble in polar solvents, while the hydrophobic group functions when Ag-NMC attacks hydrophobic cell membranes.

According to Clarkson's toxicity criteria, the toxicity is divided into non-toxic when the LC_{50} value is above 1000 g/mL, low toxic when the LC_{50} value is 500 - 1000 g/mL, moderately toxic when the LC_{50} value is

100 - 500 g/ml, very toxic when the LC_{50} value is 100 - 500 g/ml. LC_{50} 0 - 100 g/ml (Hamidi et al., 2014). The results showed that the LC_{50} values for chitosan and NMC were 2189.89 mg/L and 1499.64 mg/L, respectively. According to Clarkson's toxicity criteria, chitosan and NMC are non-toxic. Meanwhile, Ag-NMC is toxic because it has an LC_{50} value of 945,492 mg/L. Increased toxicity may result from Ag ion release. Silver NPs and silver ions contribute to toxicity (Siddiqi et al., 2018).

Antimicrobial Activity Studies

Diffusion method

The results of antimicrobial activity using the agar diffusion method can be seen in **Figure 7**. Ag-NMC has a larger diameter of inhibition zone than NMC, chitosan, and 1% acetic acid. The Chitosan solvent used in this study was acetic acid. Acetic acid also provides antibacterial activity (Fraise et al., 2013). **Figure 7** shows the antibacterial activity of *S. aureus* is greater than the antibacterial activity of *E. coli*. Gram-positive bacteria tend to be more sensitive to antibacterial components. This is due to the relatively simpler structure of the cell wall of gram-positive bacteria, making it easier for antibacterial compounds to enter the cells and find targets to work. Gram-positive bacteria have a cell wall made entirely of peptide polyglycogen. The peptidoglycan layer is made up of a network of pores, which allows foreign molecules to enter the cell easily and absorb ions more readily. While Gram-negative bacteria are surrounded by a thin layer of peptide polyglycogen, and their outer layer is composed of lipopolysaccharides, lipoproteins, and phospholipids (Mohamed et al., 2013). Gram-negative bacteria typically have more complicated cell walls.

When an acidic environment exists, amine groups ($-NH_2$) in chitosan will be converted into a $-NH_3^+$ group, so that the chitosan has a polycationic charge. This polycationic charge will interact with microorganisms. These electrostatic interactions result in: (1) changes in the permeability properties of the membrane walls. Consequently, the microorganism growth is inhibited by causing an internal osmotic imbalance, and (2) Microorganisms release

electrolytes, including potassium ions, and protein components due to the hydrolysis of peptidoglycans in their walls (Mohamed et al., 2013). In NMC, one H atom in the amine group is replaced by $-CH_3$, which is an electron-releasing group. This results in spreading the positive charge in NMC so that the cation is more stable. Therefore, the antimicrobial activity of NMC is more prominent than that of chitosan. The antimicrobial activity of Ag-NMC is always greater than that of NMC and chitosan. This is due to the influence of metal Ag on NMC. The more Ag in NMC, the greater the number of positive charges, the greater the interaction with the negative charge on the bacteria. Silver ions will affect denaturation while also interacting with thiols from proteins, causing the inactivation of bacterial proteins (Franci et al., 2015).

Chitosan's antifungal mechanism encompasses all wall morphogenesis with chitosan molecules that disrupt fungal growth. Chitosan is very effective in inhibiting spore germination, elongation of the germ tube, and It spreads inside hyphae by upsetting the action of the enzymes answerable for fungal development. (Mohamed et al., 2013).

Determination of MIC and MBC of *E. coli* bacteria

Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial agent that can inhibit microbial growth. The addition of samples of Ag-NMC, NMC, and chitosan can inhibit the growth of bacteria. This is shown by measuring the optical density (OD) of the UV-Vis spectrophotometer at a wavelength (λ) of 600 nm. **Figure 8** shows the higher the sample concentration, the lower the OD. Low OD indicates less turbidity from bacteria. The TPC results in **Table 1** show the MIC values for Ag-NMC, NMC and chitosan are 100 ppm, 100 ppm, and 300 ppm, respectively. The smaller the MIC value, the greater the antibacterial activity (Mohamed et al., 2013). Minimum Bactericidal Concentration (MBC) is the lowest concentration of antimicrobial agents that can kill 99.95% of microorganisms in the inoculum (Pansara et al., 2019). Based on **Table 2**, the MBC values for Ag-NMC, NMC, and chitosan are 140 ppm, 160 ppm, and 340 ppm, respectively.

Table 1. The number of *E. coli* colonies on the determination of the MIC values

Test Solution (ppm)	The number of colonies (10^4 CFU/mL)		
	Ag-NMC	NMC	Chitosan
5	2.70	56.33	-
10	1.30	20.33	-
50	0.33	14	158.33
100	0.20	2.33	27.33
200	0	0	10.33
300	-	-	1
400	-	-	0

* Remarks: (-) not tested

Table 2. The number of *E. coli* colonies on the determination of the MBC values

Test Solution (ppm)	The number of colonies (10^4 CFU/mL)		
	Ag-NMC	NMC	Chitosan
120	0.025	6.67	-
140	0	3.33	-
160	0	0	-
180	0	0	-
320	-	-	0.33
340	-	-	0
360	-	-	0
380	-	-	0

* Remarks: (-) not tested

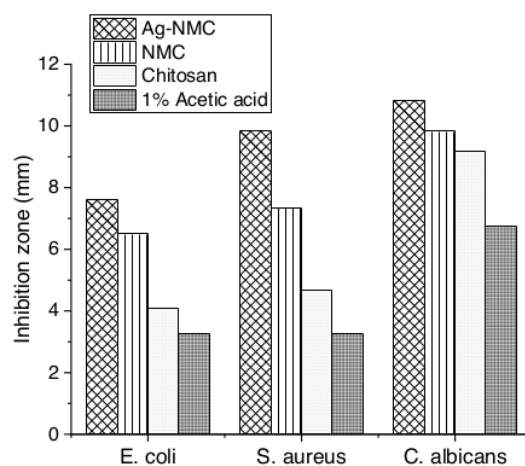
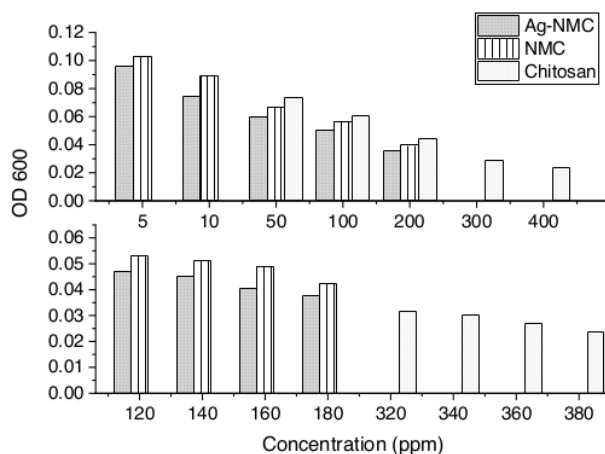
**Figure 7.** The inhibition zones**Figure 8.** Inhibition effect of different concentrations samples on the growth of *E. coli* in LB medium.**Determination of MIC and MBC of *S. aureus* bacteria**

Figure 9 shows that the greater the concentration of Ag-NMC, NMC, and chitosan, the greater the inhibition of growth of *S. aureus* bacteria. The absorbance value of *S. aureus* is lower than that of *E.*

coli. This shows that the antibacterial activity of Ag-NMC, NMC, and chitosan against *S. aureus* bacteria is greater than that of *E. coli* bacteria. Several studies have stated that chitosan provides greater antibacterial activity against gram-positive than gram-

negative bacteria (Mohamed et al., 2013; Goy et al., 2016).

The TPC results obtained can be seen in **Table 3** and **Table 4**. The MICs of Ag-NMC, NMC, and chitosan are 50 ppm, 100 ppm, and 100 ppm, respectively. The MIC value for Ag-NMC is greater than the MIC synthesized by other researchers (Shanmugam et al., 2016). MBC Ag-NMC, NMC, and chitosan are at 70 ppm, 140 ppm, and 160 ppm, respectively. These results indicate that the antibacterial activity against *S. aureus* bacteria from

Ag-NMC is twice that of NMC. Likewise, Ag/chitosan provides antibacterial activity against *S. aureus* bacteria twice that of chitosan (Kumar-Krishnan et al., 2015). MIC and MBC values to inhibit *S. aureus* bacteria are lower than *E. coli* bacteria. This result is proportional to the diameter of the inhibition zone of diffusion and absorbance where *S. aureus* bacteria can be more inhibited than *E. coli* bacteria. Other researchers also stated that chitosan had lower MIC and MBC values of *S. aureus* bacteria than *E. coli* bacteria (Fernandes et al., 2010).

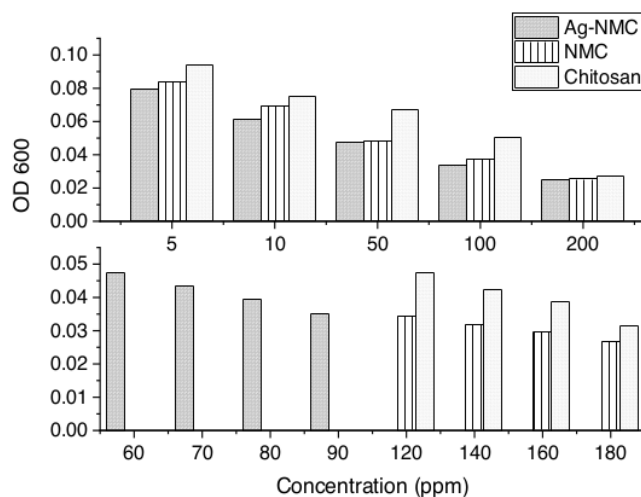


Figure 9. Inhibition effect of different concentrations samples on the growth of *S. aureus* in LB medium.

Table 3. The number of *S. aureus* colonies on the determination of the MIC values

Test Solution (ppm)	The number of colonies (10^4 CFU/mL)		
	Ag-NMC	NMC	Chitosan
5	1.1	147.67	500
10	0.53	15.37	80.5
50	0.067	8.6	50
100	0	1.67	10
200	0	0	0

Table 4. The number of *S. aureus* colonies on the determination of the MBC values

Test Solution (ppm)	The number of colonies (10^4 CFU/mL)		
	Ag-NMC	NMC	Chitosan
60	0.025	-	-
70	0	-	-
80	0	-	-
90	0	-	-
120	-	0.033	0.067
140	-	0	0.033
160	-	0	0
180	-	0	0

* Remarks: (-) not done

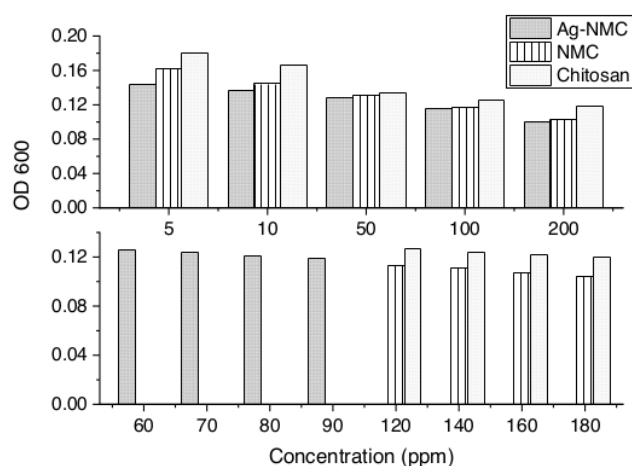


Figure 10. Inhibition effect of different concentrations samples on the growth of *C. albicans* in SD medium.

Table 5. The number of *C. albicans* colonies on the determination of the MIC values

Test Solution (ppm)	The number of colonies (10^4 CFU/mL)		
	Ag-NMC	NMC	Chitosan
5	3.12	970.6	2280
10	1.82	361	928
50	0.53	50	197.33
100	0	2.39	7.17
200	0	0	0

Table 6. The number of *C. albicans* colonies on the determination of the MFC values

Test Solution (ppm)	The number of colonies (10^4 CFU/mL)		
	Ag-NMC	NMC	Chitosan
60	0,067	-	-
70	0	-	-
80	0	-	-
90	0	-	-
120	-	0,033	0,082
140	-	0	0,035
160	-	0	0
180	-	0	0

* Remarks: (-) not done

Determination of MIC and MFC of *C. albicans* fungi

Figure 10 shows that the greater the concentration of Ag-NMC, NMC, and chitosan, the greater the ability to inhibit the growth of the fungus *C. albicans*. The determination of MIC and MFC values can be seen in **Table 5** and **Table 6**. The MIC values of Ag-NMC, NMC, and chitosan are 50 ppm, 100 ppm, and 100 ppm, respectively. The minimum values of the fungicidal concentration (MFC) of Ag-NMC, NMC, and chitosan are 70 ppm, 140 ppm, and 160 ppm, respectively. From the results of the determination of MIC and MFC, it is concluded that the antifungal activity of Ag-NMC is greater than that of NMC and

chitosan. The MFC value of Ag-NMC as a result of this study is lower than the MFC value of Ag-chitosan for *C. albicans*, which is 100 ppm (Kulatunga et al., 2017). Ag-chitosan can cause cell membrane disruption (Kulatunga et al., 2017).

CONCLUSION

Modification of chitosan to Ag-NMC could reduce MW and increase solubility. Ag-NMC is toxic in contrast to chitosan and NMC which are non-toxic. A positive correlation between the concentration of Ag-NMC, NMC, and chitosan particles with antimicrobial activity was proven, which was indicated by a decrease

in the OD value. Antimicrobial activity was different between *S. aureus* bacteria, *E. coli* bacteria, and *C. albicans* fungi. The antimicrobial activity of Ag-NMC, NMC, and chitosan against *S. aureus* was greater than that of *E. coli*. The MIC values for *S. aureus* bacteria, *E. coli* bacteria, and *C. albicans* fungi were 50 ppm, 100 ppm, and 50 ppm, respectively. MBC Ag-NMC values against *S. aureus* and *E. coli* bacteria were 70 ppm and 140 ppm, respectively. The MFC values against the fungus *C. albicans* were 70 ppm, 140 ppm, and 70 ppm, respectively. The smaller the MIC value, the greater the antimicrobial activity. It is hoped that Ag-NMC can be widely applied as an antimicrobial agent.

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