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# Preface: The 16th Joint Conference on Chemistry 2021 (16th JCC)

AIP Conference Proceedings **2553**, 010001 (2022); <https://doi.org/10.1063/12.0012151>

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## *The 16<sup>th</sup> Joint Conference of Chemistry*

*"Visualizing the latest up-front of chemistry research  
and education for better future"*

**VIRTUAL CONFERENCE**  
**SEPTEMBER 9, 2021**

### **Preface: The 16<sup>th</sup> Joint Conference on Chemistry 2021 (16<sup>th</sup> JCC)**

The Joint Conference on Chemistry is an annual conference organized by the consortium of Chemistry Department of five universities in Central Java that are: Sebelas Maret University (UNS), Diponegoro University (UNDIP), Semarang State University (UNNES), Jenderal Soedirman University (UNSOED), and Satya Wacana Christian University (UKSW). The joint conference was established in 2006. In 2014, the conference scope was enhanced to be International Conference by inviting participants worldwide. In 2020 the Covid-19 pandemic occurred, so that the conference was held in virtual conference mode, and so does this 16<sup>th</sup> JCC. However, we are confident that implementing the virtual conference does not defeat the conference's purpose as a forum for sharing research and collaboration between researchers in chemistry and all related fields. The theme of the 16th Joint Conference in Chemistry 2021 is "Visualizing the latest upfront of chemistry research and education for a better future." This conference aims to provide a forum for scientific discussion, professional networking, research collaboration, education, and dissemination of scientific research, innovation, and industrial products in the scope of chemistry, chemical science, and education and the related fields.

This edition of AIP Conference Proceedings is a collection of 50 selected papers presented during the 16<sup>th</sup> JCC. The articles cover various conference topics and represent the state of the art and perspectives of chemistry research. The submissions received two or more reviews, and the scientific committee thoroughly analyzed the score and comments of the reviewers to put together a complying collection. The committees would like to thank all the authors who contributed to the conference and the present issue and the reviewers who provided invaluable expertise to our work.

Chair of the 16<sup>th</sup> JCC  
Anung Riapanitra



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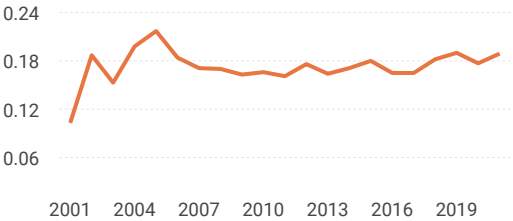
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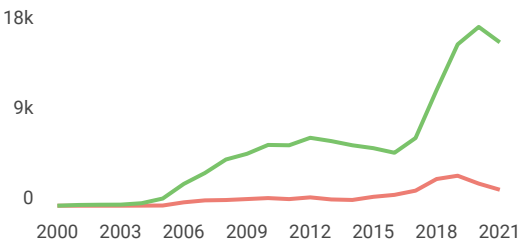


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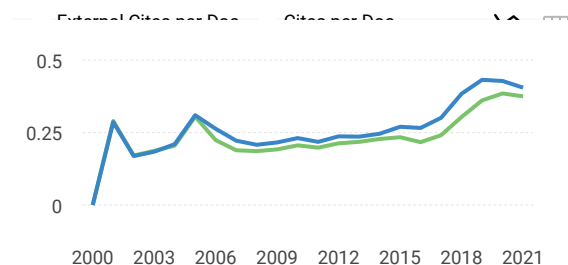
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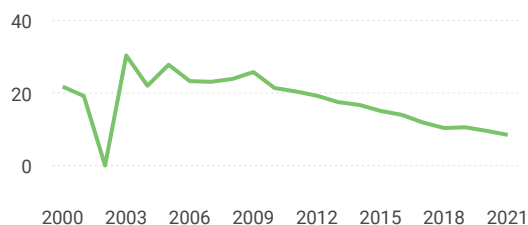


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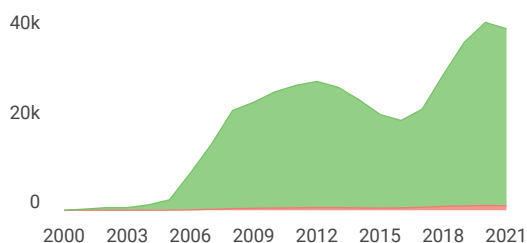
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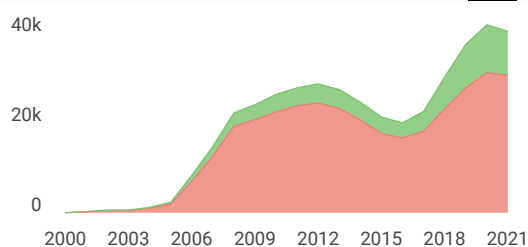
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reply

**Melanie Ortiz** 1 month ago

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A **Aizat Akmal A.Mohamad Beddelee** 7 months ago

Hi, may I know if this journal is still covered by Scopus Index as the time this comment is written June 2022, the coverage expired already.

Thanks

reply



**Melanie Ortiz** 7 months ago

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R **RATHEESH KUMAR NM** 9 months ago

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reply



**Melanie Ortiz** 9 months ago

SCImago Team

Dear Ratheesh,  
Thank you for contacting us.  
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Best Regards, SCImago Team

V **Viktor V.Chistyakov** 10 months ago

Dear Scimago!  
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Thank You  
Best regards  
V.Ch.

reply



**Melanie Ortiz** 10 months ago

Dear Viktor,  
Thank you for contacting us. Please see comments below.  
Best regards, SCImago Team

D **Dr Kumutha D** 1 year ago

Dear Sir,  
Greetings!!!!

What is the Q value? Because, in our institution Q1, Q2, Q3 is very much necessary...  
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Thanks

reply



**Melanie Ortiz** 1 year ago

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Best regards, SCImago Team

F **Fathi awad** 2 years ago

Hi  
I am asking about the Q factor for AIP proceedings.

Thank you very much

reply



**Melanie Ortiz** 2 years ago

Dear Fathi,  
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G **Ghazwan Jreou** 2 years ago

Dear sir  
respects  
would you please let me know in which Q is your journal (conferences and proceeding) classified?

according to Scopus Q classification list .  
with regards.

reply



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K **Kay** 2 years ago

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R **Ruslan** 2 years ago

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reply



**Melanie Ortiz** 2 years ago

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V **Vikas** 2 years ago

currently, the journal is not assigned quartile (Q indexing). When we can expect the assignment.

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**Melanie Ortiz** 2 years ago

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S **Siddik** 3 years ago

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H **Hassan Yassein** 3 years ago

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reply



**Melanie Ortiz** 3 years ago

SCImago Team

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**Khairil** 3 years ago

Is this proceeding ranked Q4?

reply

A **ali mohammed** 3 years ago

why this journal dont have any rank yet ?  
it is dont belong to Q1,2,3,4 ?

reply



**Melanie Ortiz** 3 years ago

SCImago Team

Dear Ali,  
Thank you for contacting us. We calculate the SJR data for all the publication types, but the Quartile data are only calculated for Journal type's publications. Best regards,  
SCImago Team

A **Akshya Sekar** 3 years ago

Hi mam/sir,

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Thanks

reply



**Melanie Ortiz** 3 years ago

SCImago Team

Dear Akshya,

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D **Duha Ahmed** 3 years ago

dear Admin

about the AIP Conference Proceeding can you see the Scopus site because the date end to 2019 is there any update about this time or change it to 2020 in the near future and you will see it in the site of Scopus

<https://www.scopus.com/sourceid/26916>

I hope the AIP Conference Proceeding is still in the Scopus for 2020

with my best wishes

Miss Duha

reply



**Melanie Ortiz** 3 years ago

SCImago Team

Dear Duha,

Thank you for contacting us. Unfortunately, we cannot see what will happen in the future with this journal. Best Regards, SCImago Team

M

**mohammed** 3 years ago

Is the ( AIP Conference Proceeding ) out of Scopes because I tried to search for it in Scopes and I did not find it  
Please answer me

reply



**Melanie Ortiz** 3 years ago

SCImago Team

Dear Mohammed,

thank you for contacting us. You can find it in Scopus:  
<https://www.scopus.com/sourceid/26916>

Best Regards, SCImago Team

T

**Thanh Quang Khai Lam** 3 years ago

Dear Elena Corera!

Can you tell me "Lecture notes in civil engineering" in Q4?

i don't see in Scimago.

Thank you

reply



**Melanie Ortiz** 3 years ago

SCImago Team

Dear Thanh,

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SCImago Team

T

**Teo Jin Chuan** 3 years ago

Dear Admin,

Can i know is this journal Q1,Q2,Q3 or Q4. Thank you.

Regards

reply



**Melanie Ortiz** 3 years ago

SCImago Team

Dear Teo, thank you very much for your request. You can consult that information in SJR website. Best Regards, SCImago Team

H **Hassan Abdulhadi** 4 years ago

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reply

H **Hassan Abdulhadi** 4 years ago

I ASKE ABOUT AIP CONFERENCE PROCEEDINGS WITHIN SCOPUS OR THOMSON REUTERS WITH BEST WISHES



**Elena Corera** 4 years ago

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Dear Hassan,

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SCImago Team

T **Tarik** 4 years ago

Dear. Elena  
Hi

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journal of AIP conference proceedings .

Best regards

TArIk AlOmran

reply



**Elena Corera** 4 years ago

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D **Dunia** 4 years ago

dear

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reply



**Elena Corera** 4 years ago

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Best Regards,  
SCImago Tea

B **Budi Adiperdana** 4 years ago

Dear Admin,

Could you please add the Quartile Rank for AIP Conference Proceedings

Best regards,  
Budi

reply



**Elena Corera** 4 years ago

SCImago Team

Dear Budi, for Conferences and Proceedings the SJR is not calculated. Best Regards,  
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# Antimicrobial Activity of N-Methyl Chitosan and Correlation with Their Degree Substitution

Mardiyah Kurniasih<sup>1, a)</sup>, Purwati<sup>1, b)</sup>, Intan Romadhani<sup>1 c)</sup>, and Ratna Stia Dewi<sup>2, d)</sup>

<sup>1</sup> Chemistry Department, Faculty of Mathematics and Natural Sciences, Jenderal Soedirman University, Jl. Dr. Soeparno, Purwokerto 53123, Indonesia

<sup>2</sup> Mycology & Phitopathology Laboratory, Faculty of Biology, Jenderal Soedirman University, Jl. Dr. Soeparno, Purwokerto 53123, Indonesia

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<sup>b)</sup> ppurwati@gmail.com

<sup>c)</sup> intan.romadhani@gmail.com

<sup>d)</sup> ratna.dewi0509@unsoed.ac.id

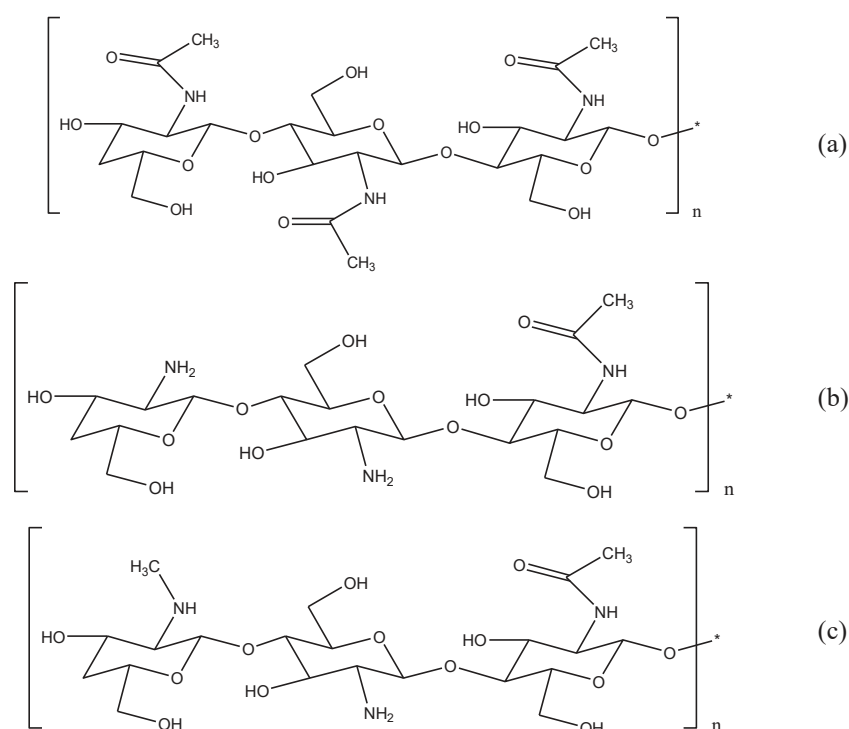
**Abstract.** N-methyl chitosan is obtained from amination reductive reaction. N-methyl chitosan is polycationic and has the ability to damage cell walls in microbes. The number of moles of chitosan, moles of formaldehyde and the reaction temperature can affect the characteristics of N-methyl chitosan such as the degree of substitution and antimicrobial activity. The purpose of this study was to determine the relationship between the degree of substitution (DS) of N-methyl chitosan with the antimicrobial activity on *E. coli* and *S. aureus* as well as on *C. albicans*. In this study, the synthesis of N-methyl chitosan was carried out with variations of moles of chitosan, moles of formaldehyde and the reaction temperature to obtain N-methyl chitosan with 9 different characteristics. N-methyl chitosan, the synthesized products, was tested for its antimicrobial activity on *S. aureus* bacteria, *E. coli* bacteria and *C. albicans* fungi. Antimicrobial activity was seen from the largest inhibition zone formed, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). From the IR analysis, it was found that 9 types of N-methyl chitosan gave different DS values. The antimicrobial test results showed that N-methyl chitosan with different DS values had different antimicrobial activities. Overall, N-methyl chitosan showed better antimicrobial activities than chitosan.

**Keywords:** *C. albicans* fungi, *E. coli* bacteria, N-methyl chitosan, *S. aureus* bacteria

## INTRODUCTION

Chitin is the second most abundant biopolymer in the world after cellulose. Chitin can be found in the shells of crustaceans [1] such as shrimp [2] and Mollusca [3]. Chitosan or poly (1,4)-2-amine-2-deoxy- $\beta$ -D-glucose can be obtained from deacetylation of chitin. Chitin deacetylation process can be carried out using enzymatic methods or chemical methods [4]. Chitin deacetylation process using an acid solution is rarely carried out, because the glycosidic bond in chitin is very susceptible to the addition of acid so that in general the chitin deacetylation reaction uses an alkaline solution such as NaOH.

Chitosan is a biopolymer consisting of D-glucosamine and N-acetyl-glucosamine units linked by  $\beta$ -(1-4) glycosidic bonds. Alkyl derivatives can be obtained by reductive alkylation method using ketones or aldehydes to produce imine bases with the reducing agent sodium borohydride or cyanoborohydride [5]. The amino group in chitosan causes chitosan to be polycationic. The positive partial charge on chitosan is able to attract the partial negatively-charged molecules strongly. This causes chitosan to be more active and to have many benefits. Chitosan has the ability to inhibit the growth of microbes, such as gram-positive and gram-negative bacteria [6, 7] as well as fungi and candida [2]. The ability of chitosan to inhibit microbes comes from positively-charged amino groups, so that it can damage the cell walls of microbes. Figure 1 shows the chemical structure of chitin and chitosan.



**FIGURE 1.** Chemical structure of (a) chitin, (b) chitosan, (c) N-methyl chitosan [8]

Chitosan has a weakness that it is not easily soluble in water and some organic solvents because chitosan has a rigid crystalline structure due to the presence of intramolecular and intermolecular hydrogen bonds. Therefore, it is necessary to modify chitosan to increase the solubility of chitosan. N-methyl chitosan is a chitosan derivative from the alkylation reaction of chitosan using a reductive amination reaction, so that the nitrogen in chitosan undergoes an electrophilic substitution reaction. Several N-alkyl chitosan derivatives have been investigated for their antimicrobial activity, including N-methyl chitosan [9, 10], trimethyl chitosan and diethyl methyl chitosan [9], carboxymethyl chitosan [9, 11], N-guanidinium chitosan acetate and N-guanidinium chitosan [12]. Modification of chitosan to N-alkyl chitosan will increase the solubility of chitosan [10], antimicrobial activity [9, 12], antioxidant activity [13] and increase cholesterol adsorption [8]. The reaction temperature, the ratio of moles of chitosan to moles of formaldehyde will affect the characteristics of N-methyl chitosan, one of which is the degree of substitution. The purpose of this study was to determine the relationship between the degree of substitution of N-methyl chitosan with antimicrobial activity against *Escherichia coli* bacteria, *Staphylococcus aureus* bacteria, and *Candida albicans* fungi.

## MATERIALS AND METHODS

### Reagents and materials

Chitin powder purchased from CV Pratama (Indonesia) were used, NaOH, NaCl, CH<sub>3</sub>COOH, NaBH<sub>4</sub>, CH<sub>2</sub>O 37%, H<sub>2</sub>SO<sub>4</sub> 98%, and Sabouraud Dextrose Agar (SDA) were obtained from Merck. Luria Bertani Agar (LBA) was purchased from Culgenex, Luria Bertani Broth (LBB) was purchased from Himedia, Sabouraud Dextrose Broth (SDB) was purchased from Criterion, the microorganisms used were *E. coli* ATCC 35218 bacteria, *S. aureus* ATCC 25923 bacteria, and *C. albicans* InaCC Y1574 fungi. Two spectrophotometers were used in this research: spektrophotometer FTIR 8201PC (Shimadzu), and spektrophotometer UV-Vis (A & E Lab).

## Chitin Deacetylation

Chitin deacetylation procedure refers to the previous study [2]. Chitin was added with 60% NaOH solution with a weight/volume ratio of 1:10. The mixture was then stirred for 3 hours at a temperature of 120 °C. The resulting product was allowed to stand until a precipitate is formed. The precipitate obtained was separated and washed with distilled water until neutral. The precipitate was dried at a temperature of 40 °C.

## Synthesis of N-methyl chitosan

The procedure for the synthesis of N-methyl chitosan was modified from a previous study [10]. The 1% (w/v) chitosan solution was added with 0.1 M formaldehyde with various mole ratios of chitosan and formaldehyde (Table 1). The mixed solution was stirred for 1 hour at various temperatures (Table 1). 1 M NaOH solution was added until the pH of the solution was 4.5. A total of 2.6 mL of 10% NaBH<sub>4</sub> (w/v) was added to the solution, then stirred for 1.5 hours at a temperature appropriate to the temperature variations used (room temperature, 50 °C, and 70 °C). The pH was adjusted to reach 10 using 1 M NaOH. The precipitate was filtered and washed with distilled water, then dried at a temperature of 40 °C.

TABLE 1. N-methyl chitosan sample variation code

Reaction temperature	Mol ratio chitosan:formaldehyde		
	2:1	1:1	1:2
room temperature	NMC-A	NMC-D	NMC-G
50 °C	NMC-B	NMC-E	NMC-H
70 °C	NMC-C	NMC-F	NMC-I

## Synthetic characterization

The synthesized chitosan and N-methyl chitosan were identified using FTIR to determine the characteristic absorptions of chitosan and N-methyl chitosan. N-methyl chitosan which the greatest microbial activity was then characterized using PSA to determine the particle size, characterization by SEM to see its morphology and its molecular weight was measured.

IR spectra were also used to calculate the degree of deacetylation (DD) of chitosan and N-methyl chitosan and the degree of substitution (DS) of N-methyl chitosan. DD was calculated using baseline *b* [14] using the following equation:

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

DS was calculated from the difference between DD chitosan and N-methyl chitosan [15], according to the following equation:

$$DS = \frac{(DD \text{ Chitosan}) - (DD \text{ N-methyl chitosan})}{100}$$

Note:

DD = degree of deacetylation

A<sub>1655</sub> = absorption peak at 1655 cm<sup>-1</sup> from the amide group

A<sub>3450</sub> = absorption peak at 3450 cm<sup>-1</sup> from the hydroxyl group

DS = degree of substitution

## Antimicrobial activity test

As much as 20 mL of sterile solid media was prepared in a petri dish. As much as 200 µL (concentration 2 x 10<sup>8</sup> CFU/mL according to Mc. Farland standard) of microbes were added and leveled with drugalsky (sterile). A 10-mm well in the middle of the petri dish was made so that in each well 50 µL of sample solution with a concentration of 100 ppm could be added. Incubation was carried out at the room temperature for 24 hours, then the inhibition zone was calculated. The diameter of the largest inhibition zone among several samples of N-methyl chitosan (Table. 1) was the most optimum sample as an antimicrobial. The sample was then determined for the MIC and MBC values.

### **The minimum inhibitory concentration (MIC) test**

2 mL of sterile liquid media in a test tube was added with 2 mL of bacteria (concentration  $2 \times 10^8$  CFU/mL according to Mc. Farland standards). The solution was homogenized with a vortex for 1 minute. The solution was added with 2 mL of sample with concentrations of 50, 100, 150, 200, 250, 300, 350, and 400 ppm. The solution was homogenized by vortex and incubated at the room temperature for 24 hours. The result of incubation was taken and diluted with sterile distilled water (dilution solution  $10^{-1}$  to  $10^{-5}$ ). The solution was homogenized by vortex again for 1 minute. A total of 100  $\mu$ L of each dilution solution was put into a petri dish containing 15 mL of sterile solid media, then leveled using drugalsky (sterile), then incubated at the room temperature for 24 hours. After incubation, the number of bacteria could be calculated using the Total Plate Count (TPC) method.

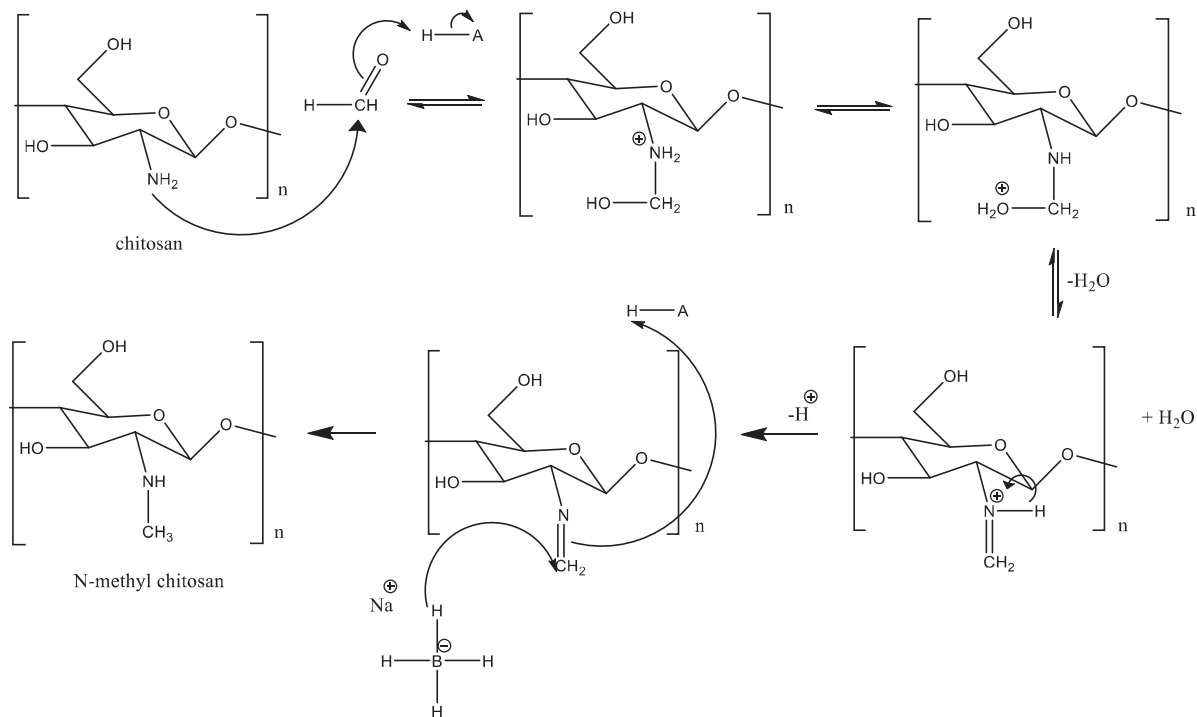
### **Uji Minimum Bactericidal Concentration (MBC)**

The preset MIC values and concentrations were used to determine the range in the MBC assay. The sample concentration with the least in the absence of bacterial growth, was considered the minimum killing concentration of bacteria.

## **RESULTS AND DISCUSSION**

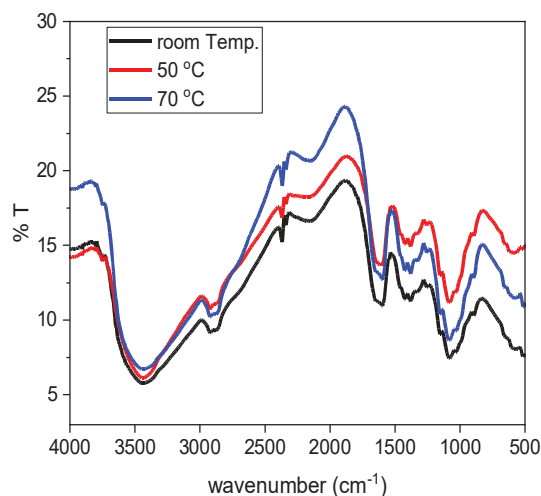
### **Characteristics of chitosan and N-methyl chitosan**

Chitosan is produced from the chitin deacetylation process. The deacetylation process in this study was carried out by adding an alkaline solution, namely NaOH solution. The addition of NaOH aimed to break the covalent bond of the acetyl group on the acetamide to form an amino group. Chitosan has low solubility. The addition of methyl groups into chitosan could increase the solubility of chitosan [16]. The reaction mechanism for the formation of N-methyl chitosan is presented in Fig 2. The methyl group of formaldehyde would replace one hydrogen atom in the amino group. After the addition of aldehydes, it was necessary to control the pH in the formation of imine [17]. Generally, imines will form at pH 4 and 5. The solution in an acidic state could protonate the carbonyl group so that the carbonyl C atom in formaldehyde had more electrophilic properties so that it was able to attack  $\text{NH}_2$  in chitosan. If the conditions are too acidic, then  $\text{NH}_2$  would be protonated to become  $\text{NH}_3^+$  causing a loss of nucleophilicity in the addition of formaldehyde in the initial reaction, while if it was too alkaline then the -OH group would be difficult to protonate to  $-\text{OH}_2^+$  so that the water elimination reaction to form iminium at a later stage could not proceed. The addition of  $\text{NaBH}_4$  had a function as a reducing agent that could change the double bond at  $\text{C}=\text{N}$  to  $\text{C}-\text{N}$  [10]. The imine compound would be formed when it was reduced by sodium borohydride [15]. N-methyl chitosan precipitate would be formed after the pH of the solution was changed to 10.

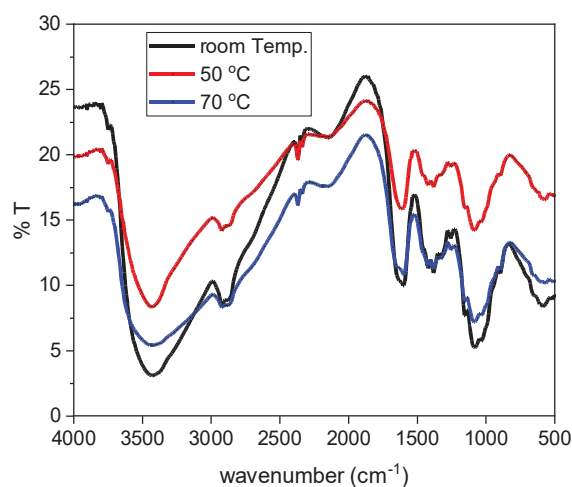


**FIGURE 2.** The reaction mechanism for the formation of N-methyl chitosan

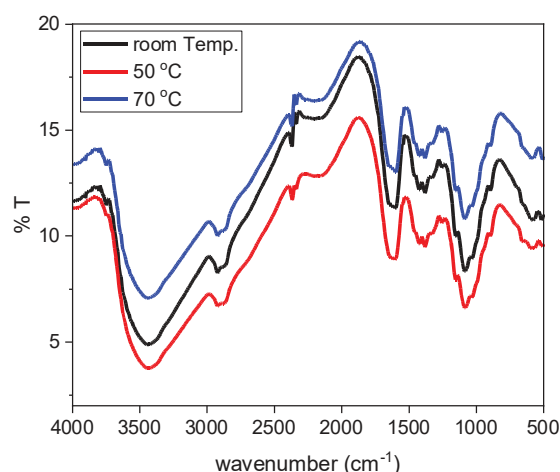
To see the relationship between DS and antimicrobial activity, in this study, various conditions for the synthesis of N-methyl chitosan were carried out. Variations were made for the addition of formaldehyde and temperature. The amount of formaldehyde might affect the degree of substitution (DS) while the temperature would affect the rate of reaction. In this study, 9 variations of N-methyl chitosan were produced which were synthesized under different conditions. Physically, N-methyl chitosan powder was not much different, all of it was brownish in color. The IR spectrum of the synthesized N-methyl chitosan is presented in Fig 3 to 5.



**FIGURE 3.** IR spectra of N-methyl chitosan on mole ratio of chitosan to moles of formaldehyde (2:1)



**FIGURE 4.** IR spectra of N-methyl chitosan on mole ratio of chitosan to moles of formaldehyde (1:1)

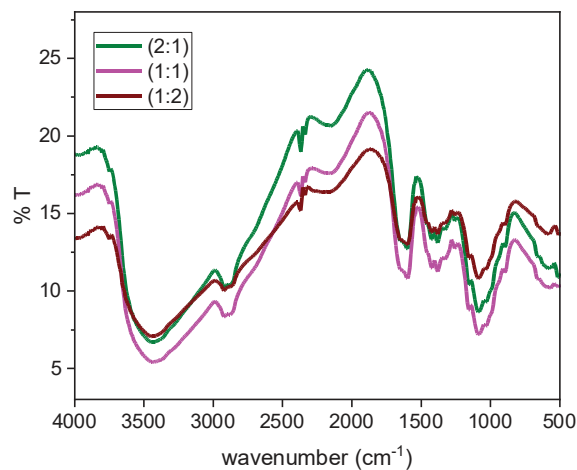


**FIGURE 5.** IR spectra of N-methyl chitosan on mole ratio of chitosan to moles of formaldehyde (1:2)

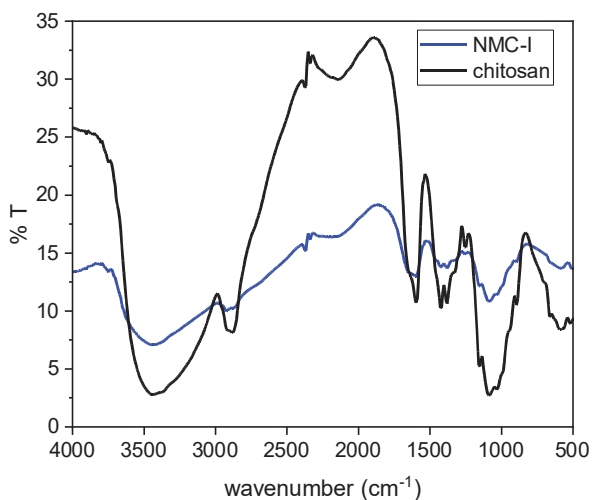
Figure 3 to 5 shows an increase in temperature resulting in a change in the intensity of the -NH absorption. N-methyl chitosan which was synthesized with the ratio of moles of chitosan: moles of formaldehyde of 2:1 and 1:2 had the highest absorption intensity values of -NH with a reaction temperature of 70 °C.

Spectrum results of N-methyl chitosan at various mole ratios of formaldehyde (2:1; 1:1; and 1:2) at a temperature of 70 °C are presented in Fig 6. Figure 6 shows that the highest absorption intensity of -NH was found in NMC-I, namely N-methyl chitosan which was synthesized with a ratio of moles of chitosan: moles of formaldehyde of 1:2. The peak intensity of -NH absorption was affected by the addition of a methyl group to the amino group causing a change in the dipole moment of N-methyl chitosan.

The difference between the IR spectra of chitosan and N-methyl chitosan is presented in Fig 7. Chitosan and N-methyl chitosan gave absorption at a wavenumber of 3448 cm⁻¹ which was a strain vibration of -NH and -OH. Absorption at 2924 cm⁻¹ indicated the -CH strain. In N-methyl chitosan, it showed an absorption peak at a wavenumber of 1597 cm⁻¹ indicating that the -NH₂ group of chitosan had been converted to -NH with one alkyl substituent. The asymmetric absorption of the methyl group was shown at the wavenumber 1381 cm⁻¹. The intensity of the peak strain on the uptake of -NH in chitosan was smaller than in N-methyl chitosan.

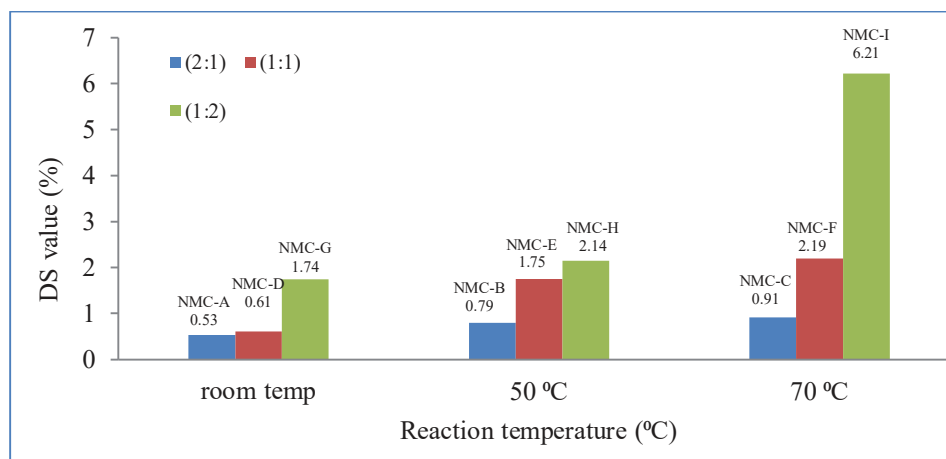


**FIGURE 6.** IR spectrum on the variation of the mole ratio of formaldehyde at a reaction temperature of 70 °C



**FIGURE 7.** IR spectra of chitosan and N-methyl chitosan

The IR spectra of chitosan and N-methyl chitosan were also used to determine the degree of deacetylation (DD) of chitosan and the degree of substitution (DS) of N-methyl chitosan. The DD value of chitosan produced was 84.705%. The DS value of N-methyl chitosan was affected by the mole ratio of chitosan and mole of formaldehyde used during the synthesis. Relationship of DS value on variation of N-methyl chitosan is presented in Fig 8. NMC-I (N-methyl chitosan synthesized with a mole ratio of 1:2 chitosan:mole formaldehyde at 70 °C) had the largest DS value.



**FIGURE 8.** Relationship of DS value on variation of N-methyl chitosan

### Antimicrobial activity of N-methyl chitosan

The results of the diffusion test on samples of acetic acid, chitosan and variations of N-methyl chitosan to determine the antibacterial activity of *E. coli* and *S. aureus* are presented in Table 2. Chitosan and N-methyl chitosan showed the ability to inhibit the growth of *E. coli* bacteria, *S. aureus* bacteria and *C. albicans* fungi. This is similar to the results of previous studies [9]. The diameter of the inhibition zone of NMC-I was 8.92 mm in *S. aureus*, 11.58 mm in *E. coli*, and 4.67 in *C. albicans*. Meanwhile, chitosan and acetic acid had a smaller diameter of inhibition zone than all variations of N-methyl chitosan. The greater the DS value of N-methyl chitosan, the greater the antimicrobial activity, indicated by the larger the diameter of the inhibition zone formed.

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**TABLE 2.** The results of the diffusion of *E. coli* and *S. aureus* bacteria

Samples	Diameter of Inhibition Zone (mm)		
	<i>E. coli</i> bacteria	<i>S. aureus</i> bacteria	<i>C. albicans</i> fungi
Acetic acid	0.42	0.33	0.17
Chitosan	0.67	0.50	0.33
NMC-A	6.17	2.50	1.83
NMC-B	7.75	6.08	2.67
NMC-C	8.67	7.42	3.33
NMC-D	6.75	3.67	2.08
NMC-E	8.08	6.58	2.92
NMC-F	8.83	8.50	4.08
NMC-G	7.41	5.00	2.33
NMC-H	8.33	7.25	3.17
<b>NMC-I</b>	<b>11.58</b>	<b>8.92</b>	<b>4.67</b>

Minimum inhibitory concentration (MIC) is the lowest antimicrobial concentration that will inhibit the growth of microorganisms after incubation for one night [18]. Determination of MIC was carried out using TPC (*Total Plate Count*) calculations to determine the number of growth colonies after the addition of antibacterial substances, namely N-methyl chitosan and chitosan. The antibacterial activity of chitosan was affected by the concentration of chitosan [19]. Table 3 shows that the MIC of N-methyl chitosan (NMC-I) against *S. aureus* was found in N-methyl chitosan



with a concentration of 350 ppm. At 400 ppm concentration of N-methyl chitosan, no growth of *S. aureus* was found. While at 400 ppm chitosan, bacterial growth was still noticed.

**TABLE 3.** The number of *S. aureus* colonies in determining the MIC value

Test Solution (ppm)	Number of Colonies (CFU/mL)	
	N-methyl chitosan	Chitosan
0	478.00 x 10 <sup>5</sup>	478.00 x 10 <sup>5</sup>
50	64.00 x 10 <sup>5</sup>	240.00 x 10 <sup>5</sup>
100	48.00 x 10 <sup>5</sup>	134.00 x 10 <sup>5</sup>
150	12.25 x 10 <sup>5</sup>	76.00 x 10 <sup>5</sup>
200	5.25 x 10 <sup>5</sup>	32.00 x 10 <sup>5</sup>
250	3.75 x 10 <sup>5</sup>	17.00 x 10 <sup>5</sup>
300	3.25 x 10 <sup>5</sup>	11.00 x 10 <sup>5</sup>
350	3.00 x 10 <sup>5</sup>	5.25 x 10 <sup>5</sup>
400	0	5.00 x 10 <sup>5</sup>

After obtaining the MIC for N-methyl chitosan, then the process to determine MBC (Minimum Bactericidal Concentration) was carried out. MBC is defined as the minimum concentration of antimicrobial substances that are bactericidal. Based on Table 4, it can be seen that the MBC value of N-methyl chitosan was at a concentration of 390 ppm, while at 400 ppm chitosan, the growth of *S. aureus* bacteria was still noticed. This is possible because the particle size of N-methyl chitosan is smaller than that of chitosan.

**TABLE 4.** The number of *S. aureus* colonies in determining the MBC value

Test Solution (ppm)	Number of Colonies (CFU/mL)	
	N-methyl chitosan	Chitosan
300	4.00 x 10 <sup>5</sup>	5.50 x 10 <sup>5</sup>
310	3.75 x 10 <sup>5</sup>	5.25 x 10 <sup>5</sup>
320	3.50 x 10 <sup>5</sup>	5.00 x 10 <sup>5</sup>
330	3.25 x 10 <sup>5</sup>	4.50 x 10 <sup>5</sup>
340	3.00 x 10 <sup>5</sup>	4.00 x 10 <sup>5</sup>
350	2.75 x 10 <sup>5</sup>	2.75 x 10 <sup>5</sup>
360	2.50 x 10 <sup>5</sup>	2.50 x 10 <sup>5</sup>
370	0.50 x 10 <sup>5</sup>	2.00 x 10 <sup>5</sup>
380	0.25 x 10 <sup>5</sup>	1.50 x 10 <sup>5</sup>
390	0	0.50 x 10 <sup>5</sup>
400	0	0.25 x 10 <sup>5</sup>

Determination of MIC values in *E. coli* bacteria is presented in Table 5. Table 5 shows that the MIC in the NMC-I sample on *E. coli* bacteria was at a concentration of 300 ppm, while in chitosan it was found at a concentration of 350 ppm. Concentration of 350 ppm in N-methyl chitosan and concentration of 400 ppm in chitosan did not show any growth of *E. coli* bacteria.

**TABLE 5.** The number of *E. coli* colonies in determining the MIC value

Test Solution (ppm)	Number of Colonies (CFU/mL)	
	N-methyl chitosan	Chitosan
0	206.50 x 10 <sup>5</sup>	206.50 x 10 <sup>5</sup>
50	12.25 x 10 <sup>5</sup>	96.00 x 10 <sup>5</sup>
100	9.00 x 10 <sup>5</sup>	54.00 x 10 <sup>5</sup>
150	6.25 x 10 <sup>5</sup>	10.00 x 10 <sup>5</sup>
200	5.75 x 10 <sup>5</sup>	8.75 x 10 <sup>5</sup>
250	3.00 x 10 <sup>5</sup>	6.25 x 10 <sup>5</sup>
300	2.50 x 10 <sup>5</sup>	4.75 x 10 <sup>5</sup>
350	0	4.00 x 10 <sup>5</sup>
400	0	0

**TABLE 6.** The number of *E. coli* colonies in determining the MBC value

Test Solution (ppm)	Number of Colonies (CFU/mL)	
	N-methyl chitosan	Chitosan
300	$3.50 \times 10^5$	$4.50 \times 10^5$
310	$3.00 \times 10^5$	$4.00 \times 10^5$
320	$2.75 \times 10^5$	$3.75 \times 10^5$
330	$0.25 \times 10^5$	$3.50 \times 10^5$
340	0	$3.00 \times 10^5$
350	0	$2.50 \times 10^5$
360	0	$0.50 \times 10^5$
370	0	$0.25 \times 10^5$
380	0	0
390	0	0
400	0	0

Chitosan could affect bacterial cell membranes through electrostatic interactions, where the cationic charge of chitosan would interact with negatively-charged bacterial phospholipids. After the cell membrane was disturbed, chitosan could enter the cell and caused inhibition of DNA/RNA synthesis and disruption of protein synthesis [20]. Table 6 shows the MBC in N-methyl chitosan is a concentration of 340 ppm indicated by the absence of bacterial growth. MBC of chitosan is at a concentration of 380 ppm. MBC N-methyl chitosan obtained on *E. coli* bacteria was smaller than MBC N-methyl chitosan on *S. aureus* bacteria. *E. coli* is a type of gram-negative bacteria, while *S. aureus* is gram-positive bacteria. It has been reported that the biocidal activity for Gram-positive bacteria is greater due to the binding of chitosan to teichoic acid, thereby extracting membrane lipids. Meanwhile, in Gram-negative bacteria, chitosan will interfere with lipopolysaccharide and causes outer membrane permeability [9].

Determination of the MIC value in *C. albicans* was also carried out by determining the number of *C. albicans* colonies. Table 7 shows that the MIC of N-methyl chitosan for *C. albicans* was present at a concentration of 300 ppm, because at a concentration of 350 ppm there was no growth of *C. albicans*. The MIC for chitosan was found at a concentration of 350 ppm. The greater the concentration of chitosan and N-methyl chitosan, the greater the inhibition of microbes (*S. aureus* bacteria, *E. coli* and *C. albicans* fungi).

**TABLE 7.** The number of *C. albicans* colonies in determining the MIC value

Test Solution (ppm)	Number of Colonies	
	N-methyl chitosan	Chitosan
0	$94.00 \times 10^5$	$94.00 \times 10^5$
50	$40.00 \times 10^5$	$75.50 \times 10^5$
100	$21.75 \times 10^5$	$32.00 \times 10^5$
150	$7.00 \times 10^5$	$14.00 \times 10^5$
200	$3.75 \times 10^5$	$10.25 \times 10^5$
250	$3.25 \times 10^5$	$6.25 \times 10^5$
300	$2.75 \times 10^5$	$4.50 \times 10^5$
350	0	$4.00 \times 10^5$
400	0	0

Table 8 shows that the MBC of N-methyl chitosan for the fungi *C. albicans* is 350 ppm. KBM in chitosan is 390 ppm. The higher the concentration, the easier it was for chitosan and N-methyl chitosan to inhibit the growth of *C. albicans*. N-methyl chitosan had greater polycationic properties than chitosan. The polycationic properties of chitosan and chitosan derivatives affected the antifungal activity [21]. Chitosan and N-methyl chitosan inhibited the growth of *C. albicans* by affecting the cell wall. After the cell wall was damaged, then diffusion occurred in the hyphae, thereby interfering with the activity of enzymes responsible for fungal growth. According to Guo et al. the antifungal effect of chitosan was due to the small dispersion size, increased permeability of fungal cell membranes, and loss of cytoplasmic contents of fungal hyphae [22].

**TABLE 8.** The number of *C. albicans* colonies in determining the MBC value

Test Solution (ppm)	Number of Colonies (CFU/mL)	
	<i>N</i> -methyl chitosan	Chitosan
300	3.75 x 10 <sup>5</sup>	4.50 x 10 <sup>5</sup>
310	3.25 x 10 <sup>5</sup>	4.00 x 10 <sup>5</sup>
320	3.00 x 10 <sup>5</sup>	3.50 x 10 <sup>5</sup>
330	0.50 x 10 <sup>5</sup>	3.25 x 10 <sup>5</sup>
340	0.25 x 10 <sup>5</sup>	3.00 x 10 <sup>5</sup>
350	0	2.75 x 10 <sup>5</sup>
360	0	0.75 x 10 <sup>5</sup>
370	0	0.50 x 10 <sup>5</sup>
380	0	0.25 x 10 <sup>5</sup>
390	0	0
400	0	0

## CONCLUSION

N-methyl chitosan was successfully synthesized from chitosan through amination reductive reaction using formaldehyde. N-methyl chitosan with various mole ratios of chitosan and formaldehyde (2:1; 1:1; and 1:2) at various reaction temperatures (room temperature, 50 °C and 70 °C) had a DS value in the range of 0.53% to 6.21%. N-methyl chitosan had a better ability to inhibit microbial growth in *S. aureus* and *E. coli* bacteria and *C. albicans* fungi than chitosan. NMC-I, N-methyl chitosan which was synthesized with a mole ratio of chitosan and formaldehyde mole of 1:2, with a reaction temperature of 70 °C, gave the best antimicrobial activity. NMC-I gave antibacterial activity of *S. aureus*, *E. coli* and antifungal activity of *C. albicans* by forming maximum inhibition zones of 8.90 mm, 11.58 mm, and 4.67 mm. NMC-I showed that the MIC of *S. aureus*, *E. coli* and *C. albicans* were 350 ppm, 300 ppm and 300 ppm, respectively. The MBC of NMC-I was 390 ppm, 340 ppm and 350 ppm.

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