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Synthesis of Schiff's Base Between Dialdehid Alginate and Chitosan and Testing of Antibacterial Properties

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ABSTRACT
Schiff base synthesis has been carried out through a condensation reaction between dialdehyde alginate and chitosan. The first step is to oxidize Na-alginate using a sodium periodate (NaIO4) oxidizer, which produces alginate dialdehyde. The next step is FT-IR analysis, degree of oxidation, swelling ratio, and antibacterial properties. The FT-IR analysis results of alginate dialdehyde, which has been absorption peak at 1627.92 cm^{-1}, show stretching group C=O and wave number 1026.13 cm-,[1] stretching group COC (cyclic ether). The Schiff base has been absorption peak at 1635.64 cm^{-1}, a stretching vibration group C=N. The degree of oxidation is 33%, and the swelling ratio is 50%. The testing of antibacterial which is conducted has been antimicrobials zone of *Escherichia coli* bacteria to Schiff base 0 mm and chitosan 0.225 mm; meanwhile, for *Staphylococcus aureus* bacteria antimicrobials zone which is Schiff base 1.916 mm and chitosan 0.333 mm. It can be concluded that Schiff base can inhibit the growth of *Staphylococcus aureus* bacteria than *Escherichia coli* bacteria were not inhibited.

Keywords: Alginate Dialdehyde, Antibacterial Properties, Chitosan, Schiff Bases

ABSTRAK
Sintesis schiff base telah dilakukan melalui reaksi kondensasi antara dialdehida alginat dan kitosan. Langkah pertama adalah mengoksidasi Na-alginat dengan menggunakan oksidator natrium periodat ($NaIO_4$) yang menghasilkan alginat dialdehida. Langkah selanjutnya adalah analisis FT-IR, derajat oksidasi, rasio pembengkakan dan sifat antibakteri. Hasil analisis FT-IR untuk alginat dialdehida yang memiliki puncak serapan pada 1627,92 cm^{-1} menunjukkan gugus ulur C=O dan bilangan gelombang 1026,13 cm^{-1} menunjukkan gugus ulur C-O-C (siklik eter). Untuk Schiff base terdapat puncak serapan pada 1635,64 cm^{-1} yang merupakan vibrasi ulur gugus C=N. Tingkat oksidasi adalah 33% dan rasio pembengkakan adalah 50%. Pengujian antibakteri yang dilakukan adalah zona antimikroba bakteri *Escherichia coli* terhadap Schiff base 0 mm dan kitosan 0,225 mm; sedangkan untuk zona antimikroba bakteri *Staphylococcus aureus* yaitu Schiff base 1,916 mm dan chitosan 0,333 mm. Dapat disimpulkan bahwa Schiff base dapat menghambat pertumbuhan bakteri *Staphylococcus aureus* dibandingkan bakteri *Escherichia coli* yang tidak terhambat.

Kata Kunci: Alginat Dialdehida, Basa Schiff, Kitosan, Sifat Antibakteri

1. Introduction

Polysaccharides such as alginate can form a gel under specific conditions and function in the presence of groups such as (-OH- and –COO-). Alginate is composed of two types of blocks, namely blocks (M) (1-4)-β-D-manuronic acid and blocks (G) (1-4)-α-L-guluronic acid, and these units are in the form of homopolymers and heteropolymer chains. In connection with its ability to form gels, stabilizing properties, and high viscosity or viscosity in aqueous solutions, alginates and their derivatives are widely used in the food, cosmetic and pharmaceutical industries [1-3]

Periodate-oxidized alginates produce more reactive groups highly susceptible to faster biodegradation when used to support controlled drug delivery [4]. Therefore, it can potentially be used in several biomedical applications where essential criteria are met, namely, biocompatibility and biodegradability [5]. Alginate

oxidation can be carried out using several oxidizing agents, such as sodium meta periodate, potassium metaperiodate, and other oxidizing agents in aqueous solutions or ethanol-water mixtures. Protection of the reaction from light during oxidation is very important to limit side reactions and control the alginate oxidation level. A specific concentration of oxidant is required [16].

Chitosan is a wound healing agent with the (1-4) 2-amino-2-deoxy-β-D-glucopyranose bond, one of the most abundant polysaccharides in nature [7]. Chitosan has several excellent properties, such as biodegradable, biocompatible, non-antigenic, non-toxic, biofunctional, and antimicrobial properties, usually useful for wide use in biomedical applications, such as drug delivery materials, surgical threads, bone healing materials, especially as a desiccant wounds [8].

The presence of amine groups in chitosan can inhibit bacterial growth by binding to the negative charge of the bacterial cell wall [9]. Several studies mention the antibacterial properties of chitosan because, first, the binding of the cytoplasm by amine groups, which disrupts cell composition resulting in the death of bacteria [10], and second is the formation of a layer of chitosan on the surface of the cell membrane so that it blocks the entry and exit of fluids cells resulting in disruption of metabolism and denaturation of proteins which results in the death of bacteria.

Antimicrobial compounds affect microbial cells in several ways, namely inhibiting cell wall synthesis, inhibiting cell membrane function, inhibiting protein synthesis, and inhibiting nucleic acid synthesis [11]. Antibacterial activity is divided into two types, namely bacteriostatic activity (a condition that inhibits bacterial growth) and bactericidal activity (a condition that can kill vegetative forms of bacteria or can kill a wide range of pathogens [12].

Schiff bases are compounds consisting of imine groups or azomethine groups (-RC=N). A condensation reaction of a primary amine with an active carbonyl usually forms this Schiff base. A German researcher first synthesized this compound named Hugo Schiff in 1864. The reaction for synthesizing this Schiff base is reversible, which includes an intermediate of a carbinolamine and requires a dry atmosphere (without water), which is more often carried out by distillation with benzene to obtain the desired maximum result. The reaction is acid-catalyzed, but this catalyst is generally unnecessary when aliphatic amines are present [13].

Based on the description above, the researcher is interested in researching the Schiff base formation reaction between dialdehyde alginate and chitosan, which is tested for its antibacterial properties. FT-IR analysis was performed on sodium alginate, dialdehyde alginate, and Schiff's base samples. Furthermore, antibacterial properties were tested on *Escherichia coli* and *Staphylococcus aureus* bacteria.

2. Materials and Methods

The tools used in this study were Erlenmeyer, beaker glass, spatula, ordinary filter paper, measuring cup, hot plate, magnetic stirrer, aluminum foil, pipette, sample cup, plastic and rubber, analytical balance, burette, stative and clamps, flask measuring, funnel, petri dish, oven, desiccator, and FT-IR Spectroscopy.

The materials used in this study included: Sodium Alginate, 94.8% chitosan, NaOH pellets, aquadest, phenolphthalein indicator, HCl, NaCl, $NaIO_4$, ethanol, Hydroxylamine, PP indicator, universal indicator, *Escherichia coli* and *Staphylococcus aureus* bacteria and Mueller Hinton Agar (MHA) media.

2.1. Synthesis of Dialdehyde Alginate

As much as 2 g of sodium alginate was put into an Erlenmeyer glass coated with aluminum foil and dissolved with 80 ml of distilled water. In comparison, stirring added 2.16 g of sodium periodate. After that, the oxidation reaction was carried out by stirring for 24 hours at room temperature. Then to the oxidation results, add 4 ml of ethylene glycol and stir in the dark for 15 minutes. The product formed was purified by precipitation by adding 1 g of sodium chloride and 120 ml of ethanol. The dialdehyde alginate polymer was dissolved in 40 ml of distilled water and precipitated with 80 ml of ethanol. This process was repeated two times. The precipitate was vacuum filtered and dried at room temperature under vacuum for 24 hours and then characterized by FT-IR spectroscopy.

2.2. Oxidation Degree Determination of Dialdehyde Alginate

A total of 100 mg of the sample was dissolved in 10 ml of distilled water. 10 ml of 0.1 N NaOH was added. The solution was heated until it completely dissolved, and the solution was cooled. Added to a solution of 10

ml of 0.15 N HCl until pH < 7. Added distilled water as much as the initial volume reduced during the first heating. The solution was heated for 1 minute, then two drops of PP indicator were added. Titrate the solution with 0.1 N NaOH and then observe the color change. Determination of the degree of oxidation can be determined through the following equation:

$$\%DO = \frac{(C\ NaOH\ x\ V\ NaOH - C\ HCl\ x\ V\ HCl)}{(\frac{m}{175})}$$

2.3. Making a Schiff Base

Dialdehyde alginate is dissolved in 100 ml of distilled water with a weight of 3 g of dialdehyde alginate (3% w/v concentration). Dissolve 3 g of chitosan in 100 ml of 2% acetic acid. Then mix the dialdehyde alginate with chitosan with a volume of 6:14 each (where 6 ml of alginate dialdehyde and 14 ml of chitosan) while stirring until a hydrogel is formed. After that, it was allowed to stand for 24 hours, then the hydrogel hybrid formed was poured into a petri dish and, divided into five small parts, dried in an oven at 40°C. After drying, the FT-IR test was carried out.

2.4. Determination of Hydrogel Swelling Ratio

One part of the dry Schiff base plate was weighed, soaked in 10 ml of distilled water for 2 hours, then weighed again.

$$\%\ \text{Swelling Ratio} = (\frac{Ws - Wd}{Ws})\ \text{x}\ 100\%$$

2.5. Making Nutrient Agar Media

As much as 7 g of nutrient agar is put in an Erlenmeyer glass, dissolved in 250 ml of distilled water, and heat until all is dissolved and boils.

2.6. Preparation of Agar Media and Bacterial Culture Stock

As much as 10 ml of nutrient agar medium was put into a sterile test tube, then sterilized in an autoclave at 121°C for 15 minutes. Leave it at room temperature until it solidifies at an inclined angle of 30-45o. The bacterial culture of Staphylococcus aureus from the primary strain was taken with a bent loop needle and then inoculated on the surface of the nutrient medium to be tilted by scraping, then incubated at 35 ± 2°C for 18-24 hours. The same procedure was also done on the bacterial culture of *Escherichia coli*

2.7. Preparation of Bacterial Suspension

A total of 10 ml of distilled water was put into a test tube and then sterilized in an autoclave at 121 0C for 15 minutes. A colony of Staphylococcus aureus bacteria was taken from the bacterial culture stock with a bent loop needle, then put into 10 ml of sterile aquadest, homogenized with a vortex, and measured the absorbance of the blank was. In the form of a sterile aquadest with a wavelength of 600 nm, the absorbance value of the bacterial suspension was measured with a wavelength of 600 nm. The same thing was also done on the bacterial culture of Escherichia coli.

2.8. Preparation of Mueller Hinton Agar (MHA) Media

As much as 9.5 g of Mueller Hinton Agar powder was put in an Erlenmeyer glass, dissolved in 250 ml of distilled water, and heated until all dissolved and boiled. Then sterilized in the autoclave at 121°C for 15 minutes.

2.9. Making Nutrient Agar Media

As much as 15-20 ml of sterile Mueller Hinton Agar (MHA) media is put into a sterile petri dish until the media solidifies. A sterile cotton bud is taken, then dipped in a suspension of *Staphylococcus aureus* bacteria and rubbed onto the MHA media, which has solidified until evenly distributed. Then put the disc paper, which has been soaked with Schiff's Basa and chitosan, into the petri dish, which already contains Staphylococcus aureus bacteria, then incubate in the incubator at 35 ± 2°C for 18-24 hours. Then the diameter of the inhibition area around the test solution was measured. The same treatment was performed on *Escherichia coli* bacteria.

3. Results And Discussion

3.1 Making Dialdehyde Alginate

Dialdehyde alginate was prepared through an oxidation reaction using sodium periodate as an oxidizing agent in the dark and at room temperature and stirring for 24 hours. The oxidation reaction will occur in the –OH group at the C-2 and C-3 positions of the uronic unit of sodium alginate, as shown in the following Figure 1.

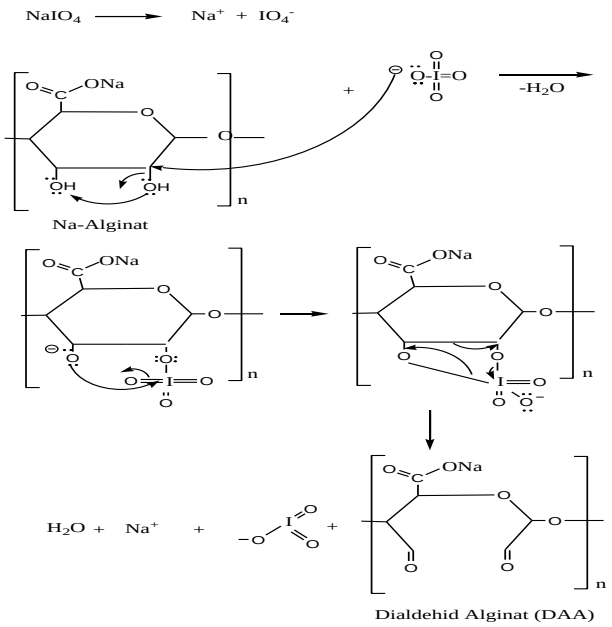

Figure 1. The oxidation reaction in the –OH group at the C-2 and C-3

This reaction will lead to the breaking or cleavage of carbon-carbon bonds (C2-C3) to form two aldehyde groups for each oxidized monomer unit so that a reactive group will be obtained along the chain and will result in a decrease in polymer stiffness which causes its rotation to increase freely. Protection of the reaction from light during oxidation is essential for limiting side reactions and controlling the degree of alginate oxidation carried out using an oxidant concentration of 2.16 g. So that in this reaction, there will be partial or partial oxidation, meaning that not all of the –OH groups present in the uronic unit are oxidized or fully oxidized. Likewise, adding ethylene glycol neutralizes sodium periodate, which does not react with sodium alginate.

3.2. FT-IR analysis of Na-Alginate

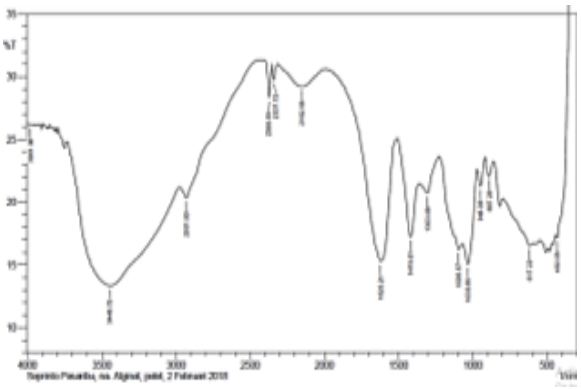

Figure 2. FT-IR analysis of Na-Alginate

Figure 2 shows the FT-IR spectrum of the sodium alginate. It showed several absorption peaks. Wave number 3448.72 cm^{-1} is the stretching vibration of the OH group, and wave number 2931.80 cm^{-1} is the

stretching vibration of the CH sp3 group. The presence of two strong absorption peaks at 1620.21 cm-1 and 1419.61 cm^{-1} is an asymmetrical and symmetrical stretching vibration of the –COO- group in the alginate polymer, together with a typical polysaccharide absorption peak at 1033.85 cm^{-1} to 1303.88 cm^{-1} from COC (cyclic ether) stretching vibrations.

3.3. Determination of the Degree of Oxidation of Dialdehyde Alginate

Determination of the degree of oxidation of dialdehyde alginate was carried out by the titration method. A NaOH solution is used to determine the degree of oxidation of the pentiter solution, where the percentage degree of oxidation is 40%. It indicates that Na-alginate has undergone oxidation to dialdehyde alginate.

3.4. FT-IR Analysis of Dialdehyde Alginatee

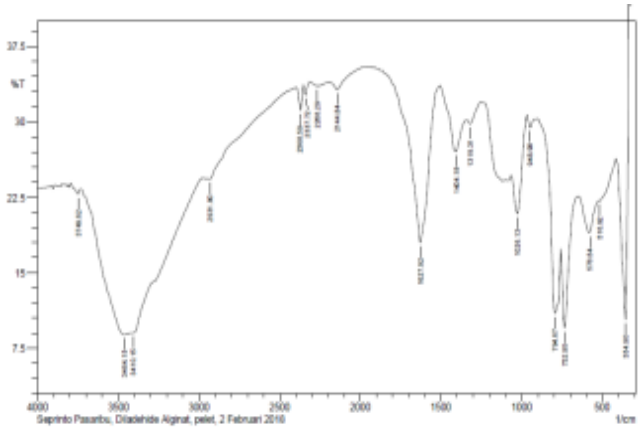

Figure .3 FT-IR Analysis of Dialdehyde Alginatee

The formation of an oxidation reaction to sodium alginate is shown by identifying changes in functional groups through analysis with FT-IR spectroscopy. Based on the FT-IR spectrum analysis results, wave number 3464.15 cm-1 is a stretching vibration of free -OH groups and intra- and intermolecular bonds that are not oxidized. The absorption peaks at 1627.92 cm^{-1} and 1404.18 cm^{-1} are the stretching vibrations of the C=O group of the dialdehyde group resulting from the oxidation process of the -OH group. The band at 1026.13 cm^{-1}, ch indicated tee COC (cyclic ether) absorption band at decreases in intensity due to chain cleavage. The absorption peaks that appear at wave numbers 794.67 cm^{-1} and 732.95 cm^{-1} are included in the CH bond, contributing to the breaking of the oxidized sodium alginate CC bond.

3.5. Determination of the Degree of Oxidation of Dialdehyde Alginate

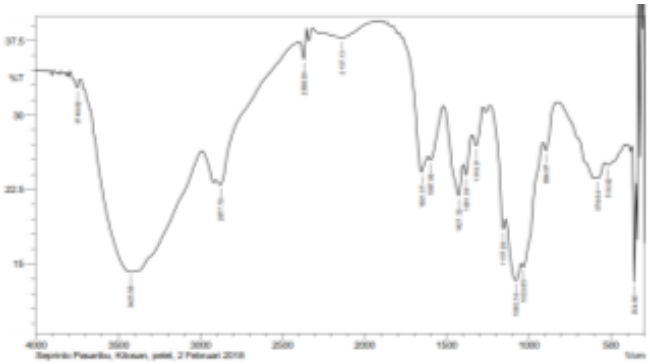

Figure .4 FT-IR Analysis of Degree of Oxidation of Dialdehyde Alginate

The results of the identification of functional groups on the FT-IR spectrum of chitosan used in this study showed several absorption peaks, namely, the absorption peak of 1651.07 cm-1 was a stretching vibration of the group (C=O in –NH-C=O), an absorption peak of 1597, 06 cm^{-1} is the stretching vibration of the NH group, the absorption peak of 1381.03 cm^{-1} is the stretching vibration of the –CH2 group, the absorption peaks of 1080.14 cm^{-1} and 1033.85 cm^{-1} are the stretching vibrations of the C-OH group.

3.6. Making of Schiff Bases

Preparation of Schiff base is carried out by reacting dialdehyde alginate with chitosan, where the N atom of the primary amine (NH_2) in chitosan, which is a nucleophile (negative), will attack the electrophile (positive), namely the C=O group of dialdehyde alginate so that the N atom will bind directly to the C atom on the carbonyl. The O atom on the carbonyl lacks electrons that bind to the H atom from NH_2 to form –OH. The N atom again has a lone pair so that the H atom attached to the N atom will attack the –OH group, which will be released as a water molecule and then form C=N (Schiff's base). The reaction can be seen in the following image.

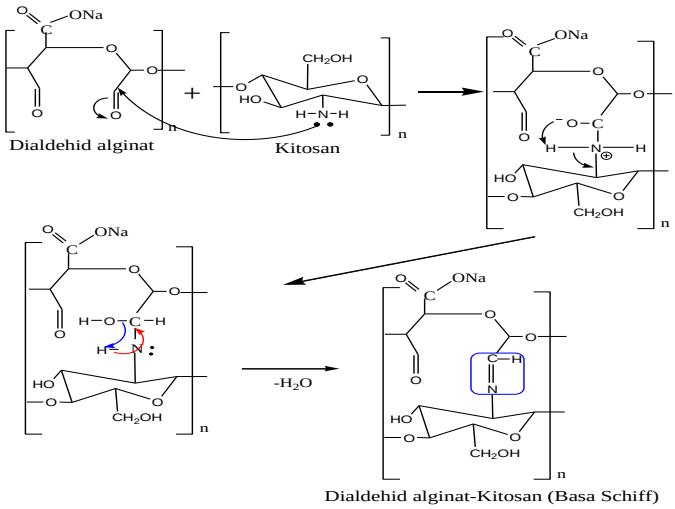

Figure 5. The *Schiff Bases mechanism*

3.7. FT-IR Analysis of Schiff Base (Dialdehyde Alginate-Chitosan)

The results of identifying functional groups on the FT-IR spectrum of Schiff's base produced in the reaction between dialdehyde alginate and chitosan are shown in the figure below.

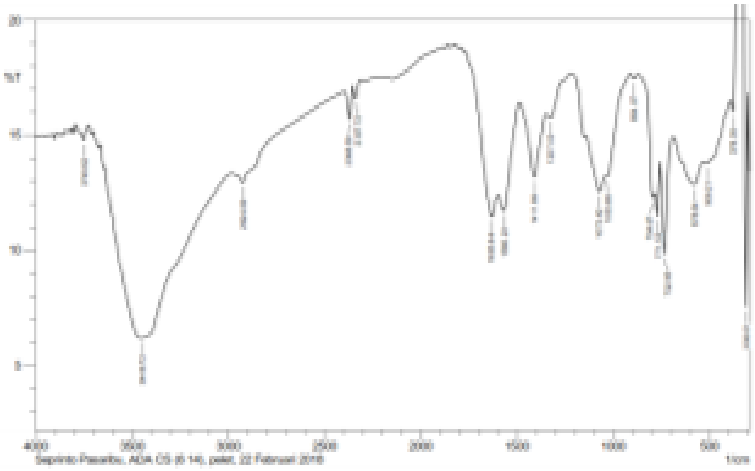

Figure 6. FT-IR Analysis of *Schiff Base (Dialdehyde Alginate-Chitosan)*

Figure 6 shows that Schiff Base (Dialdehyde Alginate-Chitosan, which indicated the wave number 3448.72 cm^{-1} is the stretching vibration of the –OH group, the wave number 2924.09 cm^{-1} is the CH stretching vibration of the aldehyde, the wave number 1635.64 cm^{-1} is the stretching vibration of the C=N group of the Schiff base, the number wave 1327.03 cm^{-1} is the stretching vibration of the $–CH_2$ group, wave numbers 1072.42 cm^{-1} and 1033.85 cm^{-1} are the stretching vibrations of the C-OH group.

3.8. Determination of Hydrogel Swelling Ratio

Determination of the degree of swelling of the hydrogel is also known as the percentage of water absorption that the hydrogel can absorb. Determination of the degree of swelling of the hydrogel was carried out by drying the hydrogel in an oven at 50°C for 24 hours, then weighing (Wd). After that, the dry sample was added with aquadest and then incubated at 37°C, then the wet sample was weighed (Ws). The percentage of swelling obtained is 50%.

3.9. Antibacterial Test

The data shown in Table 1 is obtained from the results of the antibacterial test carried out using the agar diffusion method on Schiff base samples with controls on chitosan and acetic acid against *Escherichia coli* and *Staphylococcus aureus* bacteria.

Table 1. Antibacterial test of *Escherichia coli* and *Staphylococcus aureus* bacteria.

Bacterial Isolate	Treatment	Diameter Zona Bening (nm)	Diameter of Clear Zone(nm)	Antibacterial Zone Index
Staphylococcus aureus	*Schiff Base*	17.5	6	1.916
	Chitosan	8	6	0.333
Escherichia coli	Basa Schiff	0	6	0
	Chitosan	7.35	6	0.225

Table 1 shows that as an antibacterial chitosan inhibits growth with a wide range of its target organisms. As shown in the table above, the results for *Staphylococcus aureus* bacteria was 0.333 mm, and for *Escherichia coli* bacteria, the antibacterial zone was 0.225 mm. It proves that chitosan is effective in its use as an antibacterial because the presence of amine groups in chitosan can inhibit bacterial growth by binding to the negative charge of the bacterial cell wall [8].

Meanwhile, for Schiff base, the results obtained for Staphylococcus aureus bacteria had an antibacterial zone of 1.916 mm; for *Escherichia coli* bacteria, there was no antibacterial zone. It shows that the Schiff base cannot inhibit the growth of *Escherichia coli* bacteria because the cell wall of Escherichia coli contains lipopolysaccharides, lipoproteins, and phospholipids, which trigger the activation of the immune system of these bacterial cells so that the cell walls of these bacteria cannot be broken down, compared to the cell wall of Staphylococcus aureus which does not has lipopolysaccharide so that the bacterial cell wall is easier to break down so that its growth can be inhibited [12].

4. Conclusion

Dialdehyde alginate is obtained from the oxidation reaction of sodium alginate using a sodium periodate oxidizing agent, which produces an oxidation degree of 40%. The results of FT-IR analysis at wave number 1635.64 cm-1 are the stretching vibrations of the C=N group of Schiff bases synthesized from dialdehyde alginate with chitosan, and the degree of swelling (swelling ratio) to Schiff bases is 50%. The antibacterial properties of Schiff's base on Staphylococcus aureus bacteria have an antibacterial zone of 1.916 mm and for *Escherichia coli* bacteria, an antibacterial zone of 0 mm. It shows that Schiff's base can inhibit the growth of *Staphylococcus aureus* bacteria, while *Escherichia coli* is not inhibited.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

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