

Antibacterial Activity of Patch Silver Nanoparticles and Chitosan with Cellulose Nanofibers Carriers against *Staphylococcus aureus* and *Escherichia coli*

Andre Prayoga¹, Poppy Anjelisa Zaitun Hasibuan², Yuandani²

¹Postgraduate Programs Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20115, Indonesia

²Departement of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20115, Indonesia

Abstract. One of the medical needs whose demand continues to increase is wound dressings. The wound cover must also be non-toxic, non-allergenic, made of widely available biomaterials, and have antibacterial properties that can prevent infection of the wound. Chitosan is known to have wound healing activity by acting as a blood-clotting agent and stimulating the formation of new tissue, and silver nanoparticles have good antibacterial activity. Silver Nanoparticles and Chitosan with Cellulose Nanofibers carriers (SNCCN) are made in the form of patches with the ratio formula between cellulose nanofibers and chitosan/silver nanoparticles is 1:9, 2:8, 3:8, 4:7, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0. Then the antibacterial activity was tested against *Staphylococcus aureus* and *Escherichia coli* to find the best formula for antibacterial activity. The analysis showed that the SNCCN patch with a ratio of 9:1 had the best antibacterial activity against *Staphylococcus aureus* (13.8 ± 0.05 mm) and *Escherichia coli* (12.5 ± 0.05 mm). It can be concluded that patch Silver Nanoparticles and Chitosan with Cellulose Nanofibers carriers (SNCCN) have good antibacterial activity at a concentration of 9:1 in the category of strong inhibition (10-20 mm).

Keyword: Antibacterial, Silver Nanoparticles, Chitosan, Cellulose Nanofibers, Wound Dressing.

Abstrak. Salah satu kebutuhan medis yang permintaannya terus meningkat adalah penutup luka (wound dressing). Penutup luka juga harus tidak beracun, tidak menimbulkan alergi, terbuat dari bahan biomaterial yang banyak tersedia dan memiliki sifat antibakteri yang dapat mencegah terjadinya infeksi pada luka. Kitosan diketahui memiliki aktivitas penyembuhan luka dengan bertindak sebagai agen pembekuan darah dan merangsang pembentukan jaringan baru, dan nanopartikel perak memiliki aktivitas antibakteri yang baik. Nanopartikel perak dan kitosan dengan pembawa nanoserat selulosa (NPKNS) dibuat dalam bentuk patch dengan formula perbandingan antara nanoserat selulosa dengan kitosan/nanopartikel perak adalah 1:9, 2:8, 3:8, 4:7, 5:5, 6:4, 7:3, 8:2, 9:1, dan 10:0 lalu diuji aktivitas antibakteri terhadap *Staphylococcus aureus* dan *Escherichia coli* untuk menemukan formula terbaik dalam aktivitas antibakteri. Hasil analisis menunjukkan bahwa pada patch NPKNS dengan perbandingan 9:1 memiliki aktivitas antibakteri terbaik terhadap *Staphylococcus aureus* (13.8 ± 0.05 mm) dan *Escherichia coli* (12.5 ± 0.05 mm). Dapat disimpulkan bahwa patch Nanopartikel Perak dan Kitosan dengan pembawa Nanoserat Selulosa (NPKNS) memiliki aktivitas antibakteri yang baik pada konsentrasi perbandingan 9:1 dengan kategori daya hambat kuat (10-20 mm).

*Corresponding author at: Postgraduate Programs Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20115, Indonesia.

E-mail address: andre.prayyoga@gmail.com

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1 Introduction

One of the medical needs whose demand continues to increase is wound dressings. The wound closure serves to cover the wound, stop bleeding, absorb fluid that comes out of the wound or pus, reduce pain, and provide protection for the formation of new tissue. Much research has been done to find methods of wound healing through regeneration and the use of various dressings to facilitate good wound management [1]. The ideal wound cover should be able to maintain a moist environment on the wound surface, allow gas exchange, act as a barrier for microorganisms, and remove excess exudate. The wound cover must also be non-toxic, non-allergenic, made of widely available biomaterials, and have antimicrobial properties that can prevent infection of the wound [2] because wounds on the surface of the skin are easily colonized by various kinds of organisms [3].

Nanotechnology research is developing so rapidly. Researchers continue to innovate to create nano products that are useful for society. The currently developing nanotechnology is the use of nanofibers for various products. Nanofibers have unique properties and have the potential to be applied in the fields of biology, chemistry, electronics, engineering, biomedicine, and the protection of various products. [4]-[6] One of the applications of nanofibers in the biomedical field is to heal wounds [7]. Chitosan is currently widely used in the world of food, medical, pharmaceutical, and biotechnology. In the medical world, chitosan is known to have good wound healing activity [8]. Among the antibacterial agents, silver has been known to have antimicrobial activity since ancient times to inhibit infections caused by bacteria [9] and it is known that silver and similar compounds are effective antimicrobial agents [10]. The antimicrobial activity of silver depends on the surface area and size of the silver, the larger the surface area and the smaller the size of the silver, the greater the antimicrobial activity. Therefore silver is made in the form of nanoparticles because silver nanoparticles with a greater surface area ratio have a greater antibacterial efficiency [11].

This study was conducted to test the antibacterial activity of silver nanoparticle patches and chitosan with cellulose nanofibers as carriers against *Staphylococcus aureus* and *Escherichia coli*. The use of this combination is expected to have good antibacterial power so it is expected that it will affect the speed of wound healing.

2 Materials and Methods

2.1 Material

The materials used in this study were Oil Palm Empty Fruit Bunches (OPEFB), Chitosan (Acros, China), Silver Nitrate (Emsure, German), NaOH (Emsure, German), H₂O₂ (Emsure, German), HCl (Emsure, German), HNO₃ (Emsure, German), Acetic Acid (Emsure, German), Glucose Monohydrate (Emsure, German), Muller Hilton Agar, Nutrient Broth, Gentamicin 0.1%, and distilled water.

2.2 Test Preparations

The test bacteria used for this study were *Staphylococcus aureus* and *Escherichia coli*. The method used for the isolation of cellulose fibers from OPEFB is the steam explosion method, where the OPEFB fibers that have been cleaned are mixed with 2% NaOH and soaked overnight. After that, the mixture is put into the autoclave for the steam explosion process at 130 °C with a pressure of 180 kPa for 2 hours. Then the resulting fiber is neutralized with distilled water and bleached with 10% H₂O₂ at 70°C for 3 hours, neutralized again with distilled water, and then dried in an oven at 60°C [12].

Cellulose nanofibers were obtained by hydrolyzing cellulose fibers with 10% HCL in an ultrasonicator for 2 hours and then neutralized. The result is homogenized using a high shear homogenizer at a speed of 8000 rpm for 4 hours and a suspension of cellulose nanofibers will be produced [12].

2.3 Synthesis of Chitosan and Silver Nanoparticles

The synthesis of chitosan-silver nanoparticles was carried out by mixing chitosan with reduced AgNO₃ with glucose monohydrate. 2 g of chitosan was dissolved in 100 mL of acetic acid, then reacted with 0.72 g of AgNO₃ which had previously been dissolved with 2 mL of distilled water at 95°C, the mixture was stirred for 1.5 hours, then glucose monohydrate was added in a ratio of 1:4 to AgNO₃ which was reacted for 7 hours at room temperature [13].

2.4 Transdermal Patch Preparations

Patch Transdermal preparations were prepared using variations in the concentration ratio of silver nanoparticles and chitosan with cellulose nanofibers as a combination of ingredients (Table 1.1). Each ingredient is put into a glass beaker then stirred for 30 minutes to homogenize the mixture, the mixture is poured on one print while flattening then put into an oven at 65°C for 24 hours so that a dry patch is formed, the dried patch is put into a desiccator [14].

Table 1. Transdermal patch formula is a comparison of cellulose nanoparticles and chitosan with cellulose nanofibers as carriers

Comparison of Formula Composition	F.1	F.2	F.3	F.4	F.5	F.6	F.7	F.8	F.9	F.10
Cellulose Nanofibers	1	2	3	4	5	6	7	8	9	10
Chitosan/Silver Nanoparticles	9	8	7	6	5	4	3	2	1	0

2.5 Antibacterial Activity

An antibacterial test is carried out to determine the best concentration ratio of various formulas. Nutrient media to be poured into a petri dish that is inoculated by the bacteria *Staphylococcus aureus* and *Escherichia coli*, then each patch was cut to the size of a paper disc (6 mm) and placed on a petri dish, incubation at 36-37°C for 18–24 hours. Furthermore, the zone of inhibition formed is measured using a caliper [15]. Then calculate the inhibition zone diameter.

2.6 Data Analysis

The results of the observation that the percentage of excision wound healing were statistically tested using the analysis of variance (ANOVA) test with a confidence level of 95%.

3 Result and Discussion

3.1 Antibacterial Testing

An antibacterial activity test was performed to determine the best patch ratio formula. The antibacterial test was carried out using *Staphylococcus aureus* and *Escherichia coli*. Each patch was cut into a disc paper size (6 mm) and placed on a petri dish, then incubated at 36-37°C for 18-24 hours and would produce an inhibition zone such as Figure 1 follows

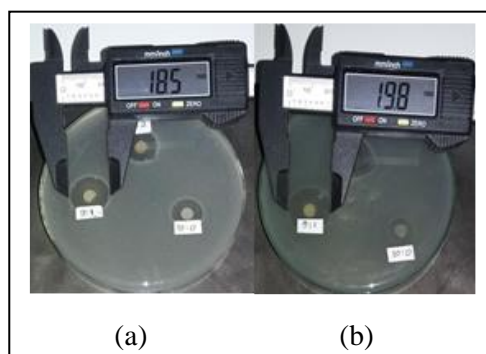


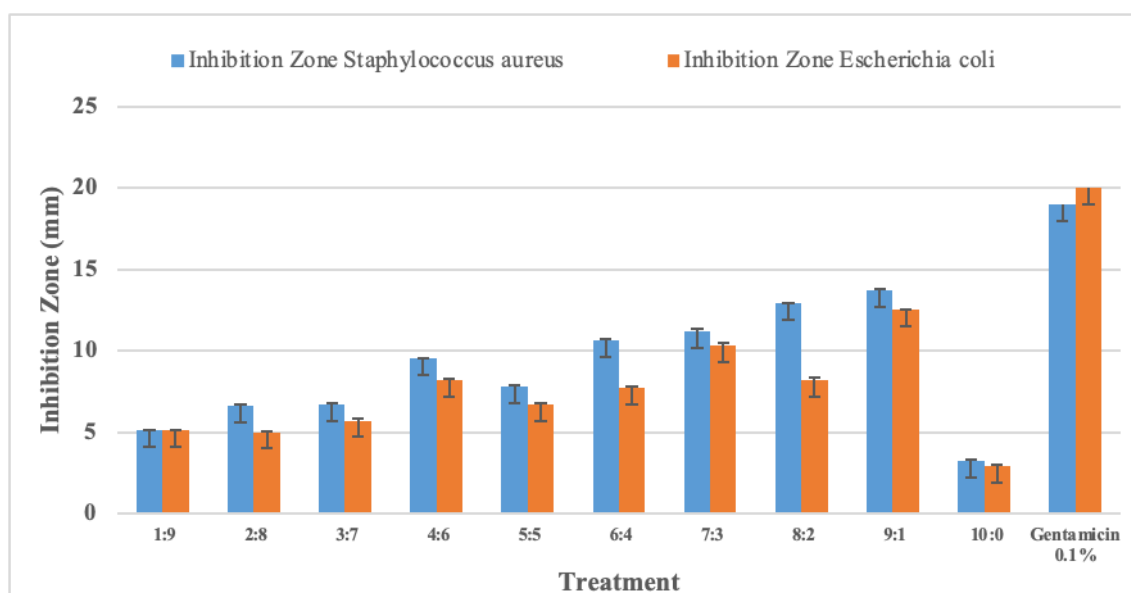
Figure 1. Antibacterial activity of SNCCN Patches against (a) *Staphylococcus aureus* and (b) *Escherichia coli* at a concentration ratio of 9:1

The antibacterial activity in Figure 1 shows the presence of a clear zone in the media from the SNCCN sample with a concentration ratio of 9:1, then calculating the diameter of the inhibition zone. The results can be seen in Table 2 below:

Table 2. Inhibition zones of various concentrations of SNCCN patch ratio against *Staphylococcus aureus* and *Escherichia coli*

Treatment	Data	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
1:9	5.1±0.05	5.1±0.05
2:8	6.6±0.11	5±0.05
3:7	6.8±0.05	5.7±0.1
4:6	9.6±0.05	8.2±0.05
5:5	7.7±0.1	6.7±0.05
6:4	10.6±0.15	7.7±0.11
7:3	11.1±0.15	10.3±0.15
8:2	12.9±0.05	8.2±0.11
9:1	13.8±0.05	12.5±0.05
10:0	3.2±0.11	2.9±0.05
Gentamicin 0.1%	19	20

Figure 2. Inhibition zone of the SNCCN patch against *Staphylococcus aureus* and *Escherichia coli*



From Figure 2, it can be seen that the concentration ratio of the 9:1 patch has the largest zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* bacteria. Based on the category of inhibitory strength, the silver nanoparticles produced have a strong inhibitory power. The category of bacterial inhibition power is classified into 4 categories, namely weak inhibition (<5 mm), moderate inhibition (5-10 mm), strong inhibition (10-20 mm), and very strong inhibition (> 20 mm) [16].

From Table 2, it can be seen that the 0.1% gentamicin antibacterial activity as a positive control has a very strong inhibitory power (>20 mm), the concentration of SNCCN patch ratio 6:4, 7:3, 8:2, and 9:1 has a strong inhibitory power (10-20 mm), the SNCCN patch ratio concentration of 1:9, 2:8, 3:7, 4:6, and 5:5 had moderate inhibition (5-10 mm), and the concentration of the SNCCN patch ratio was 10:0 as a negative control has a very weak inhibition (<5 mm).

The silver nanoparticles contained in the patch will react with the wound fluid which contains infection-causing bacteria. The silver nanoparticles will slowly release silver ions which can damage bacterial RNA and DNA, thereby inhibiting the bacterial replication process. Stunted bacterial replication will suppress bacterial growth so that wound healing will be faster [17].

Based on Table 2. Inhibition of silver nanoparticles against bacteria *Staphylococcus aureus* stronger than the inhibition of bacteria *Escherichia coli*. Bacteria *Escherichia coli* which are gram-negative bacteria have an effective permeability barrier, namely a thin layer of lipopolysaccharide on the outer membrane that can limit the penetration of the silver nanoparticle solution. Meanwhile, bacteria *Staphylococcus aureus* which are bacteria gram-positive only has a peptidoglycan layer which is more accessible for permeation by silver nanoparticles so that it is easy to penetrate and damage the bacterial cell walls [18].

3.2 Data Analysis

ANOVA data for the inhibition zone diameter of *Staphylococcus aureus* and *Escherichia coli*, showed a significant value of 0.132 ($p > 0.05$), which means that the data were normally distributed, there was no significant difference in the effect of the treatment given on the tested bacteria. This shows that the positive control and ten concentration SNCCN patch ratios are good at the ratio of 1:9, 2:8, 3:8, 4:7, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0 have provided an activity that inhibits the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria.

The analysis showed that the SNCCN patch with a ratio of 9: 1 had the best antibacterial activity against *Staphylococcus aureus* (13.8 ± 0.05 mm) and *Escherichia coli* (12.5 ± 0.05 mm) with the strong category (10-20 mm).

4 Conclusion

In this study, it can be seen that the patch Silver Nanoparticles and Chitosan with Cellulose Nanofibers carriers (SNCCN) have good antibacterial activity at a concentration of 9:1 in the category of strong inhibition (10-20 mm).

REFERENCES

- [1] H. D, Meilanny; B, Pranjono; D, Metode elektrospining untuk mensintesis komposit berbasis alginat-polivinil alkohol dengan penambahan lendir bekicot, vol. 17, no.

- November, pp. 65–71, 2015.
- [2] S. prastiana Dewi, *Perbedaan efek pemberian lendir bekicot (Achatina fulica) dan gel bioplacentonTM terhadap penyembuhan luka bersih pada tikus putih*. 2010.
- [3] N. T. Matsuura; B, Infections in patients with diabetes, *Diabetologe*, vol. 14, no. 3, pp. 136–137, 2018, doi: 10.1007/s11428-018-0331-1.
- [4] T. Subbiah, G. S. Bhat, R. W. Tock, S. Parameswaran, and S. S. Ramkumar, Electrospinning of nanofibers, *J. Appl. Polym. Sci.*, vol. 96, no. 2, pp. 557–569, 2005, doi: 10.1002/app.21481.
- [5] K. Wei *et al.*, Development of electrospun metallic hybrid nanofibers via metallization, *Polym. Adv. Technol.*, vol. 21, no. 10, pp. 746–751, Oct. 2010, doi: <https://doi.org/10.1002/pat.1490>.
- [6] N. Kimura, H. Kim, B.-S. Kim, K. Lee, and I.-S. Kim, Molecular Orientation and Crystalline Structure of Aligned Electrospun Nylon-6 Nanofibers: Effect of Gap Size, *Macromol. Mater. Eng.*, vol. 295, Dec. 2010, doi: 10.1002/mame.201000235.
- [7] D. A. Soscia, N. A. Raof, Y. Xie, N. C. Cady, and A. P. Gadre, Antibiotic-Loaded PLGA Nanofibers for Wound Healing Applications, *Adv. Eng. Mater.*, vol. 12, no. 4, pp. B83–B88, Apr. 2010, doi: <https://doi.org/10.1002/adem.200980016>.
- [8] T. A. Khan, K. K. Peh, and H. S. Ch'ng, Reporting degree of deacetylation values of chitosan: The influence of analytical methods, *J. Pharm. Pharm. Sci.*, vol. 5, no. 3, pp. 205–212, 2002.
- [9] M. Gajbhiye, J. Kesharwani, A. Ingle, A. Gade, and M. Rai, Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole, *Nanomedicine Nanotechnology, Biol. Med.*, vol. 5, no. 4, pp. 382–386, 2009, doi: <https://doi.org/10.1016/j.nano.2009.06.005>.
- [10] F. Mirzajani, H. Askari, S. Hamzelou, M. Farzaneh, and A. Ghassempour, Effect of silver nanoparticles on *Oryza sativa* L. and its rhizosphere bacteria, *Ecotoxicol. Environ. Saf.*, vol. 88, pp. 48–54, 2013, doi: <https://doi.org/10.1016/j.ecoenv.2012.10.018>.
- [11] I. Ristian, S. Wahyuni, and I. Supardi, Kajian Pengaruh Konsentrasi Perak Nitrat Terhadap Ukuran Partikel Pada Sintesis Nanopartikel Perak, *IJCS - Indones. J. Chem. Sci.*, vol. 3, no. 1, 2014.
- [12] S. Gea, D. Andita, S. Rahayu, D. Y. Nasution, S. U. Rahayu, and A. F. Piliang, Preliminary study on the fabrication of cellulose nanocomposite film from oil palm empty fruit bunches partially solved into licl/dmac with the variation of dissolution time, *J. Phys. Conf. Ser.*, vol. 1116, no. 4, pp. 0–7, 2018, doi: 10.1088/1742-6596/1116/4/042012.
- [13] A. Amin, N. Khairi, and E. Allo, Sintesis dan karakterisasi kitosan dari limbah cangkang udang sebagai stabilizer terhadap Ag nanopartikel, *Fuller. J. Chem.*, vol. 4, no. 2, p. 86, 2019, doi: 10.37033/fjc.v4i2.100.
- [14] A. Arifin and M. Iqbal, Formulasi dan Uji Karakteristik Fisik Sediaan Patch Ekstrak Etanol Daun Kumis Kucing (*Orthosiphon stamineus*), *J. Ilm. Manuntung*, vol. 5, no. 2, pp. 187–191, 2019.
- [15] D. E. Putri Mambang, R. -, and D. Suryanto, AKTIVITAS ANTIBAKTERI EKSTRAK TEMPE TERHADAP BAKTERI *Bacillus subtilis* DAN *Staphylococcus aureus*, *J. Teknol. dan Ind. Pangan*, vol. 25, no. 1, pp. 115–118, 2014, doi: 10.6066/jtip.2014.25.1.115.
- [16] W. W. Davis and T. R. Stout, Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error., *Appl. Microbiol.*, vol. 22, no. 4, pp. 659–665, 1971, doi: 10.1128/aem.22.4.659-665.1971.
- [17] H. A. Ariyanta, PREPARASI NANOPARTIKEL PERAK DENGAN METODE REDUKSI LUKA INFEKSI Silver Nanoparticles Preparation by Reduction Method and its Application as Antibacterial for Cause of Wound Infection, pp. 36–42.
- [18] A. L. Prasetiowati *et al.*, Indonesian Journal of Chemical Science Sintesis Nanopartikel Perak dengan Bioreduktor Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L .) sebagai Antibakteri, vol. 7, no. 2, 2018.