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Antifungal Activity of Patch Silver Nanoparticles and Chitosan with Cellulose Nanofibers Carriers against *Trichophyton rubrum* and *Pitysporum ovale*

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Abstract. Wounds that are not treated and are kept open will provide an entrance for microorganisms from outside that can cause infection. One of the medical needs whose demand continues to increase is wound dressings. Chitosan is known to have wound healing activity by stimulating the formation of new tissue, and silver nanoparticles have good antimicrobial activity. Silver nanoparticles and chitosan with cellulose nanofibers carrier are made in the form of patches with the ratio formula between cellulose nanofibers and chitosan/silver nanoparticles are 1:9, 2:8, 3:8, 4:7, 5:5, 6:4, 7:3, 8:2, 9:1, and 10:0 then tested the antifungal activity against *Trichophyton rubrum* and *Pitysporum ovale* for finding the best formula for antifungal activity. The analysis showed that the patch with a ratio of 6:4 had the best antifungal activity against *Trichophyton rubrum* (14.7±0.1 mm) and a 9:1 patch on the *Pitysporum ovale* (6.9±0.05 mm) gave a significant difference to negative control ($p<0.05$). It can be concluded that the Patches Silver Nanoparticle and Chitosan with Cellulose Nanofibers (SNCCN) carriers have good antifungal activity in the inhibitory category.

Keyword: Antifungal, silver nanoparticles, chitosan, cellulose nanofibers, wound dressing.

Abstrak. *Luka yang tidak dirawat dan terus dibiarkan terbuka akan menjadi pintu masuk bagi mikroorganisme dari luar yang dapat menyebabkan terjadinya infeksi. Salah satu kebutuhan medis yang permintaannya terus meningkat adalah penutup luka (wound dressing). Kitosan diketahui memiliki aktivitas penyembuhan luka dengan merangsang pembentukan jaringan baru, dan nanopartikel perak memiliki aktivitas antimikroba yang baik. Nanopartikel perak dan kitosan dengan pembawa nanoserat selulosa 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 antijamur terhadap Trichophyton rubrum dan Pitysporum ovale untuk menemukan formula terbaik dalam aktivitas antijamur. Hasil analisis menunjukkan bahwa pada patch dengan perbandingan 6:4 memiliki aktivitas antijamur terbaik terhadap Trichophyton rubrum (14.7±0.1 mm) dan patch 9:1 pada Pitysporum ovale (6.9±0.05 mm) memberikan berbeda signifikan terhadap kontrol negatif (p<0,05). Dapat disimpulkan bahwa patch Nanopartikel Perak dan Kitosan dengan pembawa Nanoserat Selulosa (NPKNS) memiliki aktivitas antijamur yang baik dengan kategori daya hambat.*

Kata Kunci: *antijamuri, nanopartikel perak, kitosan, nanoserat selulosa, penutup luka.*

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1 Introduction

The skin is the largest organ that covers the entire body and functions as protection from various kinds of disturbances in the form of both physical and chemical influences so that the skin is very susceptible to trauma and injury [1]. A wound is a condition where normal anatomical functions and structures are damaged, whereas to produce improved function and anatomical continuity, a wound healing process must be carried out, which is a complex dynamic process. [2]. One type of wound is an excision wound caused by a cut of the tissue by a sharp object cut [1].

Wounds can be intentionally made for a specific purpose, such as an incision in surgery or a traumatic wound such as an accident. Wounds that are not treated and are kept open will become an entrance for microorganisms from outside which can cause infection and the wound becomes difficult to recover and can even get worse [3].

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 [4].

Nanoparticles can be developed in various fields, one of which is in the health sector. 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 [5] - [7]. One of the applications of nanofibers in the biomedical field is to heal wounds [8]. 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 [9]. Silver is a metal that can be applied in the health sector because it has antibacterial and antifungal properties [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 greater antimicrobial efficiency [11].

This research was carried out to test the antifungal activity of patches Silver Nanoparticle and Chitosan with Cellulose Nanofibers (SNCCN) carriers against *Trichophyton rubrum* and *Pitysporum ovale*. The use of this combination is expected to have good antifungal 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), H_2O_2 (Emsure, German), HCl (Emsure, German). , HNO_3 (Emsure, German), Acetic Acid (Emsure, German), Glucose Monohydrate (Emsure, German), Muller Hilton Agar, Nutrient Broth, Ketokonazole 2%, and distilled water.

2.2 Fungals

The test fungals used for this study were *Trichophyton rubrum* and *Pitysporum ovale*.

2.3 Test Preparations

The method used for the isolation of cellulose fibers from OPEFB is the steam explosion method, total of 75 g of powdered OPEFB powder was then put into a beaker glass and 1 L of 2% NaOH solution was added. Put it in the autoclave and the pressure was set at 130 kPa and the temperature was 130ºC for 1 hour. Suddenly removed the pressure and removed the OPEFB fibres from the autoclave. And washed with water until a neutral pH. Furthermore, it was bleached using 1 L of a solution mixture consisting of 200 mL 17.5% NaOH, 200 mL 17.5% CH3COOH, 600 mL 1.75% NaOCl, and heated at 80ºC. Filtered and washed the filtrate until neutral pH. The resulting α-cellulose was dried in an oven at 60ºC and then weighed.15 minutes. Sudden pressure relief. Adjusted again to the pressure of 130 kPa for 15 minutes and repeated the hydrolysis process 8 times. Cooled, filtered, and washed the residue to neutral pH. Homogenized by using high shear homogenizer with a rotation speed of 8,000 rpm for 4 hours. Suspension drying in an oven at 60ºC [12].

2. 4 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℃, the mixture was stirrer 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. 5 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 65ºC for 24 hours so that a dry patch is formed, the dried patch is put into a desiccator[14].

Table 1. Transdermal patch SNCCN formula

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

2.6 Antifungal Activity

Antifungal activity testing Trichophyton rubrum and Pitysporum ovale was carried out by using the Kirby-Bauer smear method using disc paper. This method is carried out by a procedure, namely 20 ml of Sabouraud Dextrose Agar (SDA) agar media, each is poured into a petri dish and allowed to solidify, after which 0.1 ml of inoculum is added. Trichophyton rubrum and Pitysporum ovale. The surface of the media is wiped with a loop needle until it is evenly distributed. A sterile disc-shaped patch is placed over the agar plate using tweezers. Each petri dish was incubated for 1x24 hours at 37ºC. The zone of inhibition to growth around the disc paper shows a positive test and the diameter of the inhibition zone is measured using a calliper. The distance of the paper discs from one another is 3 cm from the edge of the media by two cm [15]. Then calculate the inhibition zone diameter.

2. 7 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 Antifungal Testing

The antifungal test was carried out using *Trichophyton rubrum* and *Pitysporum ovale*. 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

(a) (b)

Figure 1. Antifungal activity of Patches against (a) *Trichophyton rubrum* at concentration of 6:4 and (b) *Pitysporum ovale* at a concentration ratio of 9:1

The antifungal activity in Figure 1 shows the presence of a clear zone in the media from the SNCCN sample with a concentration ratio of 6:4 and 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 patch SNCCN ratio against *Trichophyton rubrum* and *Pitysporum ovale*

Treatment	Data	
	Trichophyton rubrum	*Pitysporum ovale*
1:9	1.6±0.1	1.7±0.1
2:8	2.9±0.05	2.4±0.05
3:7	3.2±0.05	2.7±0.05
4:6	3.5±0.1	2.8±0.05
5:5	6.4±0.05	3.8±0.05
6:4	14.7±0.1	5.2±0.05
7:3	14.1±0.05	5.3±0.1
8:2	11.3±0.11	5.7±0.1
9:1	12.9±0.05	6.9±0.05
10:0	2.4±0.05	1.4±0.1
Ketokonazole 2%	20	20

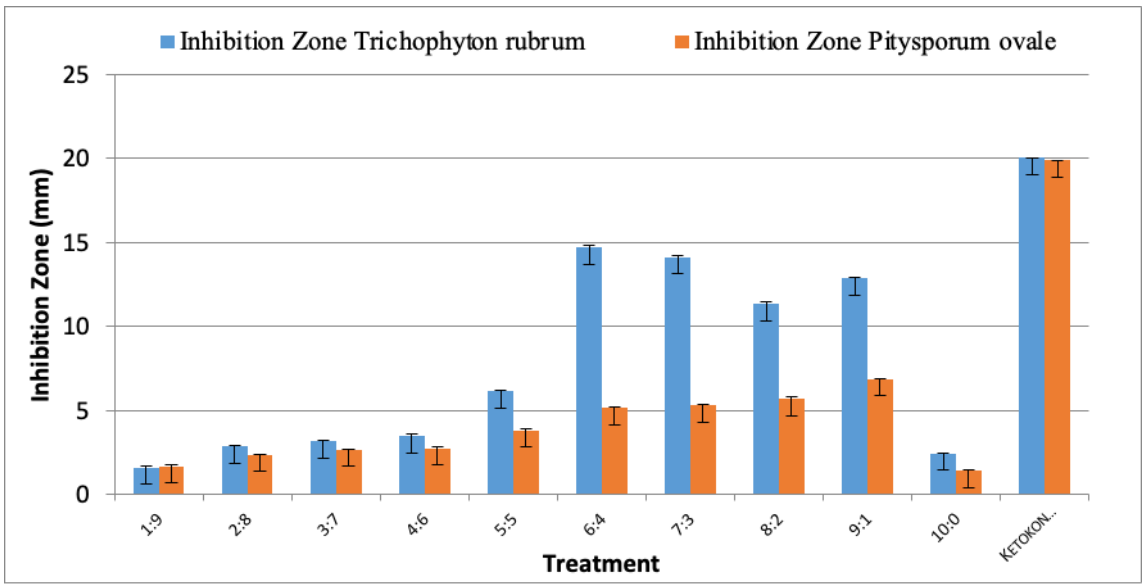

Figure 2. Inhibition zone of the patch SNCCN against *Trichophyton rubrum* and *Pitysporum ovale*

From Figure 2, it can be seen that at a concentration ratio of 6:4 patches have the greatest zone of inhibition against the fungus *Trichophyton rubrum* and at a concentration ratio of 9: 1 patch has the greatest zone of inhibition against the fungus *Pitysporum ovale*. 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 antifungal activity of ketoconazole 2% as a positive control has a very strong inhibitory power (> 20 mm), in testing for fungi. Trichophyton rubrum, concentrations of patch ratio 6:4, 7:3, 8:2 and 9:1 have strong inhibition (10-20 mm), concentration of 5: 5 patch ratio has moderate inhibition (5-10 mm), and concentration of patch ratio 1:9, 2:8, 3:7, 4:6, and 10:0 as negative controls had very weak inhibition (<5 mm). On testing for fungi *Pitysporum ovale* concentrations of 6:4, 7:3, 8:2 and 9:1 patch ratios have moderate inhibition (5-10 mm), and patch ratio concentrations of 1:9, 2:8, 3:7, 4:6, 5:5 and 10:0 as negative controls had very weak inhibition (<5 mm).

It can be seen in the table and graph that the inhibition power of silver/chitosan patches with cellulose nanofibers has a strong inhibition zone against the fungus *Trichophyton rubrum*, and the greatest inhibition is in the 6:4 patch ratio, which is equal to 14.7±0.1 mm gave a significant difference to negative control ($p < 0.05$). This shows that the silver nanoparticle patch has effectiveness as an antimicrobial agent where silver nanoparticles can adhere to the cell membrane of microorganisms so that they can interfere with cell membrane permeability and secular respiration [17]. Whereas in the fungus Pitysporum ovale the inhibition power of patches against medium microorganisms was at a ratio of 9:1 with an average diameter of 6.9±0.05 mm gave a significant difference to negative control ($p < 0.05$).

ANOVA data for the inhibition zone diameter of *Trichophyton rubrum* and *Pitysporum ovale*, showed significant difference to negative control ($p < 0.05$). which means that the data were normally distributed, there was no significant difference in the effect of the treatment given on the test mushrooms. This shows that the positive control and ten concentration 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 the fungus *Trichophyton rubrum* and *Pitysporum ovale*.

Conclusion

In this study, it can be seen that the patches of silver nanoparticles and chitosan with cellulose nanofibers carriers have good antifungal activity at a concentration ratio of 6:4 in the fungus *Trichophyton rubrum* with the category of strong inhibition (10-20 mm) and at a concentration of 9:1 in the fungus *Pitysporum ovale* with the category of moderate inhibition (5-10 mm).

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