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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SILVER NANOPARTICLES AGAINST NEISSERIA GONORRHOEAE AND CANDIDA ALBICANS

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ABSTRACT

This Research on the antibacterial and antifungal activity of nanosilver against Neisseria gonorrhoeae and Candida albincas fungi has been carried out. The purpose of this study was to determine antibacterial activity of nanosilver against Neisseria gonorrhoeae and antifungal activity against Candida albincas. Synthesis Nanosilver uses bottom up method and characterized using UV-Vis Spectrophotometer. Nanosliver concentrations used were 30, 40, 50, and 60 ppm. Antibacterial and antifungal activity tests using disk diffusion method. Observations obtained in form of the presence or absence of clear zones formed around paper discs indicate the inhibition of nanosilver on microbial growth. The results of testing the antifungal activity of Candida albicans on nanosilver with concentrations of 30, 40, 50 and 60 ppm resulted in clear zones of 9.73 nm, 11.46 nm, 11.93 nm, and 13 nm with fungal inhibition response categories is medium and strong. The results antibacterial activity test of Neisseria gonorrhoeae on nanosilver with concentrations of 30, 40, 50 and 60 ppm did not show any clear zone around the disc, it showed that nanosilver in this study did not have antibacterial activity against Neisseria gonorrhoeae.

1. INTRODUCTION

In this era of globalization there have been many advances in all fields and aspects of science about the importance of hygiene and also the basic health of physical and organ hygiene. One of the organs that really needs special care is the reproductive organs. Reproductive organs are one of the important things in every human life. Reproductive organs are the subject of several diseases. Early knowledge and understanding of the health of reproductive organs can be used as prevention for men and women so that they will be better able to maintain the health of their reproductive organs.[1]. In women, the vaginal canal is very vulnerable to risk of infection from the outside. In addition, the wrong way to clean the vagina and leave the condition of the vagina moist also triggers the disease in female reproductive organs [2]. In women, there is a term called Vaginal Infection. Vaginal infection is one of the most frequent problems where women visit a doctor [3]. One example of vaginal infection is vaginitis. Vaginitis

is inflammation and vaginal infections commonly encountered in clinical medicine [4]. Bacterial vaginosis, candidiasis vaginalis (an infection caused by *Candida* spesies) is responsible for the majority of vaginal infections in women, especially in women of reproductive age [5]. An example of the most serious disease in bacterial vaginosis is Gonorrhea disease caused by the bacterium *Neisseria gonorrhoeae* is Sexually Transmitted Disease (STD).

Gonorrhea in Indonesia ranks highest of all STD. According to the World Health Organization (WHO, 2011) as many as 70% of female patients and some male patients infected with gonorrhoeae or chlamydia have asymptomatic symptoms. Sexually transmitted diseases are also the most common cause of infertility, especially in women. Several studies in Surabaya, Jakarta and Bandung in female sex workers show that the prevalence of gonorrhoeae ranges from 7.4% - 50% [6]. Besides gonorrhea, there are also *candidiasis vaginalis* which is one of the vaginal infections caused by fungus Candida species especially *Candida albicans*. *Candida albicans* is a normal flora that lives in oral mucrosa, respiratory tract, digestive tract and in the vagina [7]. Candida albicans with excessive amounts in the body can cause disease. One example *is Candidiasis vaginalis.* Thin (1983) states that the most common cause of vaginal candidiasis is candida albicans, which is 81%, the rest is 16% by torulopsis glabarata, while the other 3% is caused by Candida tropicalis, Candida pseudotropicalis, Candida krusei and Candida stellatoidea [8]. *Candidiasis vaginalis* can occur in women of all ages, especially at puberty. The most prominent complaint in patients with vaginal candidiasis is vaginal itching accompanied by discharge of the vaginal body (fluor albus). Sometimes also found irritation, burning and dyspareunia. In the acute situation, the body of the vagina is runny while the chronic is thicker. Candidiasis that has entered the bloodstream can spread to various organs such as the kidneys, spleen, heart, brain, and cause various diseases such as endocarditis, meningitis, endophthalmitis and pyelonephritis [9].

Various attempts have been made to reduce various infectious diseases of the vagina that require antimicrobial agents to inhibit or kill bacteria and fungi that cause vaginal infections. Now many new technologies have been developed that are used to overcome problems for microbes such as bacteria and fungi. One of them is nanotechnology. Nanotechnology is the science of technology that uses the properties of molecular structures or atomic structures or materials in nanometer sizes. Nanometer-sized materials have better properties than large-sized materials. A nanomaterial or nanoparticle is a particle with the size of a nanometer (1-100 nm) [10]. One of the nanoparticles that has been developed is silver or nanosilver. Nanosilver can be obtained by synthesis using the bottom-up method, which is a synthesis process that involves chemical reactions by growing nanoparticles from a number of starting materials so that other nanometer-sized materials are produced [11].

Nanosilver has a very broad antimicrobial spectrum including antiviral activity such as HIV-AID and HSV herpes virus [12]. Nanosilver is proven to have the ability, among others, as an antibacterial and antifungal [13]. Silver nanoparticles (AgNP) also have antifungal, anti-inflammatory, antiviral, and antiplatelet activity [14]. Nanosilver as a strong antibacterial because of its chemically reactive and easily ionized nature and antimicrobial ability of nanosilver can kill all pathogenic microorganisms and there have not been reported any silver-resistant microbes [15]. The antibacterial activity of silver nanoparticles is influenced by several things, such as the concentration of silver nanoparticles, the shape of silver nanoparticles, the size of silver nanoparticles, the type of bacteria, the number of bacterial colonies and the contact time of silver nanoparticles with bacteria [16].The antibacterial ability of silver nanoparticles include damaging bacterial cell walls, disrupting cell metabolism, and inhibiting bacterial cell synthesis. Nanosilver can approach the microbial cell membrane and penetrate into the microbe. Furthermore, the nanosilver diffuses and attacks the respiratory chain of the microbes, so it can kill the microbes [17]. Nanosilver is a natural mineral that is non-toxic and safe for daily use. The combined minerals are also found in groundwater or natural water sources [18].

In this research, basic ingredients of nanosilver in the form of AgNO3 by reducing sodium citrate in aquo media [19]. Synthesis of nanosilver was carried out by bottom-up method using sodium citrate as a reducing agent. Several variations of nanosilver concentrations were performed by 30, 40, 50, 60 ppm. Nanosilver with several variations of concentration will be analyzed by UV-Vis spectrophotometer at a wavelength of 400-513 nm to determine the peak absorbance. In the antibacterial assay of Neisseria gonorrhoeae and antifungal assay of Candida albicans used disc diffusion method. The observation results obtained are the presence or absence of clear areas formed around the disc paper which shows inhibition zones on microbial growth.

2. MATERIALS AND METHODS

2.1. MATERIALS

This research was carried out using several materials including 250 ml beaker glass, 10 ml beaker glass, 50 ml beaker glass, hot plate and stirrer, Ohauss analytical balance, spatula, 100 ml measuring flask, Shimadzu UV-Vis Spectrophotometer 1800, micropipette, autoclave, incubator, calipers, ose needles, tweezers, vortex, petri dishes, AgNO3 (≥99%, Merck), sodium citrate (99%, Merck), aquades (PT Bratachem), aquabides (PT Bratachem), liquid media Potato Dextrosa Agar (DPA), ketocenazole, dimethyl sufoxide (DMSO), Neisseria gonnorhoeae stock colony, Candida albicans stock colony, filter paper, paper discs, Ciprofloxacin.

2.2. PREPARATION OF $AgNO_3$ SOLUTION

1000 ppm $AgNO_3$ solution was obtained by dissolving 1.57 g of $AgNO_3$ crystal (colored white) which was put into a 1000 ml volumetric flask, then diluted with aquabides to mark limits.Then the mixture is homogeneous and a 1000 ppm $AgNO_3$ solution is formed which is ready to be used as material in nanosilver synthesis. 1000 ppm $AgNO_3$ is used for synthesis.

2.3. SYNTHESIS OF NANOSILVER

Synthesis of nanisilver was carried out by the bottom up method by heating 200 ml of distilled water to boiling, then adding sodium gratrate 0.4 g and 1000 ppm 4 ml $AgNO_3$ solution for a concentration of 20 ppm. Warming is continued until the solution turns from colorless to yellow then grayish yellow for about 15-20 minutes. Furthermore, the colloid is cooled at room temperature. Synthesis was continued with variations of concentrations of 30, 40, 50, 60 ppm with a volume of 1000 ppm $AgNO_3$ solution of 6, 8, 10, 12 ml. The synthesized nanosilver measured its maximum wavelength on a UV-Vis spectrophotometer in the wavelength range of 400-513 nm.

2.4. ANTIBACTERIAL ASSAY OF NEISSERIA GONORRHOEAE USING DISC DIFFUSION METHOD

The steps taken in this antibacterial assay are make a suspension of the bacteria Neisseria gonorrhoeae with a turbidity of 1mc farland then insert a sterile swab into the suspension of the Neisseria gonorrhoeae and rubbed evenly into the MH plate media then saturate the disc paper in nanosilver for 15 minutes and insert the saturated disc paper into the MH plate media. The positive control used 5 mcg ciprofloxacin antibiotic disk and negative control used aquadest. Incubation at 35°-37°C for 24 hours. The results of observations can be measured use calipers.

2.5. ANTIFUNGAL ASSAY OF CANDIDA ALBICANS USING DISC DIFFUSION METHOD

The steps of make a specific culture media for Candida albicans are embed Candida albicans stock on SDB (Sabour Dextrosa Broth) media by applying 1 ose needle pure culture Candida albicans then incubated for 24 hours at 37°C. Antifungal assay use sterile paper discs dropped with 10 microliter nanosilver, then the paper discs containing nanosilver are placed on the surface of SDB media and incubated for 48 hours. The positive control used ketokenazol and negative control used aquadest. The results of observations can be measured use calipers.

3. RESULTS AND DISCUSSION

3.1. SYNTHESIS AND CHARACTERIZAION OF NANOSILVER

Synthesis of Nanosilver use bottom up method which is reducing chemicals by using sodium citrate. In synthesis of nanosilver with this method produces a change in the color of solution from initially colorless to stable yellow, so that heating can be stopped. The colorless solution indicates that there is no interaction with each other between Ag

atoms, while the stable yellow color indicates that Ag particles enter nano size and the growth of particle size (cluster) is getting bigger. The results synthesis are brownish-yellow colloids as shown in Figure 1.

Figure 1: Nanosilver colloids with concentrations of 30, 40, 50, 60 ppm

There is a difference in the intensity of the brownish yellow color that occurs in the results of nanosilver synthesis, the greater the concentration of nanosilver shows the more concentrated brownish yellow color. This happens because there are differences in the size of the cluster produced. If the concentration of nanosilver is greater then more clusters will form so that the color intensity results will be stronger. Silver atoms will interact with their fellow metal bonds and produce large numbers of nano clusters. Reaction that occurs in synthesis of nanosilver is written in equation (1).

$$4Ag^{+} + C_6H_5O_7Na_3 + 2H_2O \rightarrow 4Ag^{0} + C_6H_5O_7H_3 + 3Na^{+} + H^{+} + O_2 \quad (1)$$

Nanosilver was then characterized by a UV-Vis Spectrophotometer in the wavelength range of 300-450 nm to determine the maximum wavelength of nanosilver used to measure the cluster diameter. Cluster diameter of nanosilver was calculated using the Brush equation. The results of the characterization are shown in Table 1.

Table 1: Maximum wavelength and diameter of nanosilver cluster

Concentration of Nanosilver	λ (nm)	Diameter of nanosilver cluster
30	425	18,14
40	421	17,10
50	416	16,93
60	410	16,69

3.2. ANTIBACTERIAL ASSAY OF NEISSERIA GONORRHOEAE USING DISC DIFFUSION METHOD

Nanosilver with concentration of 30,40,50 and 60 ppm then tested for antibacterial activity against Neisseria gonorrhoeae. In this study, antibacterial assay use disc diffusion method that's make a suspension of Neisseria gonorrhoeae with a turbidity of 1mc farland then insert a sterile swab into the suspension of the Neisseria gonorrhoeae and rubbed evenly into the MH plate media then saturate the disc paper in nanosilver for 15 minutes and insert the saturated disc paper into the MH plate media. The positive control used 5 mcg ciprofloxacin antibiotic disk and negative control used aquadest. Incubation at 35°-37°C for 24 hours. The results of research using this method are the clear zone diameters that occur around the paper disk. The results of the qualitative test antibacterial activity of nanosilver against Neisseria gonorrhoeae as shown in Figure 2. and the results of the qualitative test antibacterial activity of positive control (Ciprofloxacin) against Neisseria gonorrhoeae as shown in Figure 3.

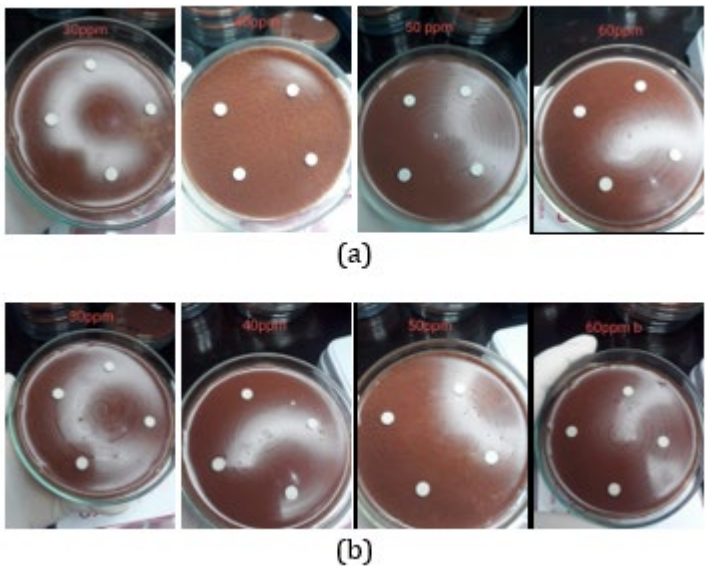

Figure 2: Result of antibacterial assay of nanosilver with concentration 30,40,50, and 60 ppm against Neisseria gonorrhoeae. (a) Replication I (b) Replication II

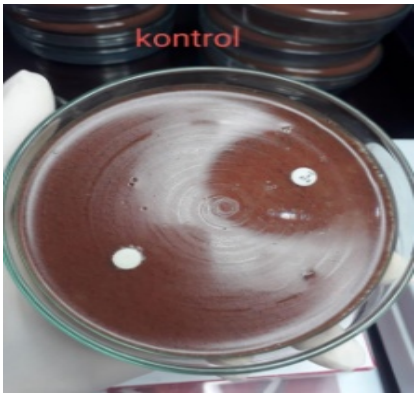

Figure 3: Results of the qualitative test antibacterial activity of positive control (Ciprofloxacin) against Neisseria gonorrhoeae

The quantitative test results of the antibacterial assay of nanosilver which is diameter of clear zone can be measured using the calipers shown in Table 2.

Table 2: Clear zone diameter of the antibacterial test on nanosilver against Neisseria gonorrhoeae

Sample	Clear zone diameter (mm)			
	I	II	III	IV
Nanosilver 30 ppm	6	6	6	6
Nanosilver 40 ppm	6	6	6	6
Nanosilver 50 ppm	6	6	6	6
Nanosilver 60 ppm	6	6	6	6
Control (+)	48	-	-	-
Control (-)	6	-	-	-

In this study, four replications were carried out and from the results of the clear zone diameter it can be stated that the nanosilver in this study does not have antibacterial activity against Neisseria gonorrhoeae, it can be known because there is no clear zone diameter that occurs around the disc paper that has been saturated on the nanosilver. The criteria for antibacterial activity according to Muharni (2016) [20] were clear zone diameters <10 nm included in the weak category, 10-16 nm included in the moderate category and >16 nm included in the strong category.

Nanosilver has good antibacterial activity but there is no research that states that nanosilver can inhibit the growth of the bacteria Neisseria gonorrhoeae. In this study, nanosilver used concentrations of 30, 40, 50 and 60 ppm and nanosilver was allowed to stand for 2 weeks after the synthesis process, after that testing was carried out on the Neisseria gonorrhoeae bacteria, it also could reduce the antibacterial properties of the nanosilver because it was not directly tested after the process synthesis is done. The absence of antibacterial activity in this study can also occur because the concentration of nanosilver used is too small or not optimal to inhibit the growth of Neisseria gonorrhoeae. N. gonorrheae is a gram-negative bacterium that has a selection system for certain substances. According to Muharini (2017) [21], the structure of gram negative bacteria also affects the results of bacterial inhibition zones because it has a more complex structure with three layers, namely the outer layer in the form of lipoprotein, the middle layer in the form of lipopolysaccharide and the inner layer in the form of peptidoglycan, which causes Gram-negative bacterial cell walls are more difficult to penetrate by antibacterial compounds and gram-negative bacteria have the property of less susceptible to some antibacterial compounds.

In negative control area (aquades) no clear zone appears. The absence of this clear zone indicates that aquades do not have antibacterial properties and can not inhibit the growth of Neisseria gonorrhoeae bacteria while in the positive control area (ciprofloxacin) shows the presence of a clear zone diameter of 48 mm which indicates that the antibiotic ciprofloxacin has a strong antibacterial ability against Neisseria bacteria gonorrhoeae.

3.3. ANTIFUNGAL ASSAY OF CANDIDA ALBICANS USING DISC DIFFUSION METHOD

Nanosilver with concentration of 30,40,50 and 60 ppm then tested for antifungal activity against Candida albicans. In this study, antifungal assay use disc diffusion method that's make a specific culture media for Candida albicans are embed Candida albicans stock on SDB (Sabour Dextrosa Broth) media by applying 1 ose needle pure culture Candida albicans then incubated for 24 hours at 37°C. Antifungal assay use sterile paper discs dropped with 10 microliter nanosilver, then the paper discs containing nanosilver are placed on the surface of SDB media and incubated for 48 hours. The positive control used ketokenazol and negative control used aquadest. The results of research using this method are the clear zone diameters that occur around the paper disk. The results of the qualitative test antifungal activity of nanosilver against Candida albicans as shown in **Figure 4.** and the results of the qualitative test antifungal activity of positive control (ketokenazol) against Candida albicans as shown in **Figure 5.**

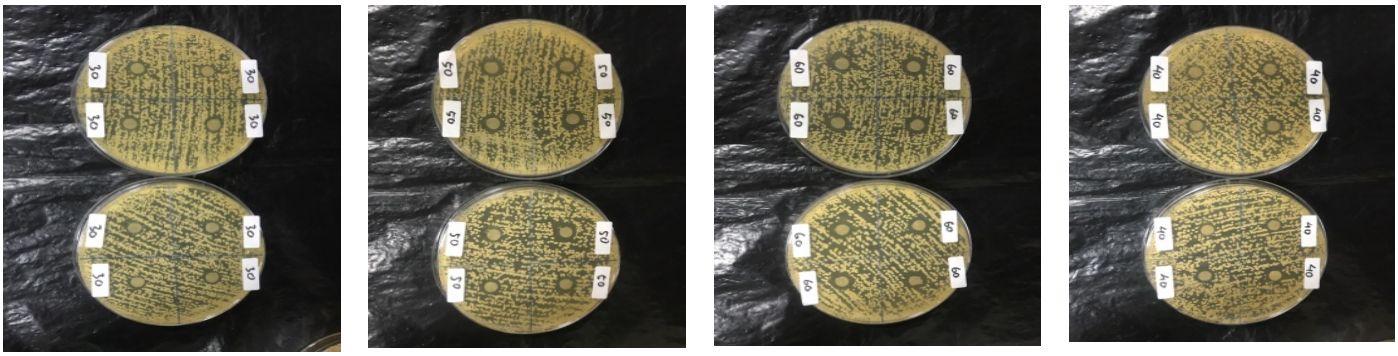

Figure 4: Result of antifungal assay of nanosilver with concentration 30,40,50,60 ppm against Candida albicans

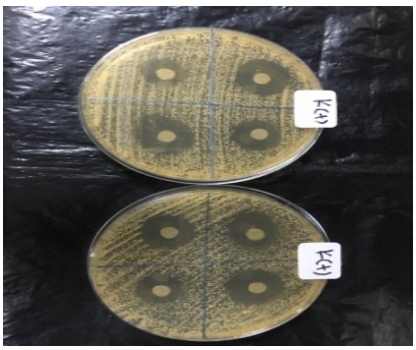

Figure 5: Result of antifungal assay of Ketokenazol against Candida albicans

The quantitative test results of the antifungal assay of nanosilver which is diameter of clear zone can be measured using the calipers shown in Table 3. and graphs of nano silver concentrations with clear zone diameters are shown in Figure 6.

Table 3: Clear zone diameter of the antifungal test on nanosilver against Candida albicans

Sample	Clear zone diameter (mm)				
	I	II	III	IV	Rata-rata
Nanosilver 30 ppm	9,70	9,85	9,85	9,0	9,73
Nanosilver 40 ppm	11,40	11,25	10,95	12,25	11,46
Nanosilver 50 ppm	11,70	13,25	10,80	11,95	11,93
Nanosilver 60 ppm	13,30	12,95	12,70	13,05	13
Control (+)	15,65	16,25	13,10	19,65	16,16
Control (-)	-	-	-	-	-

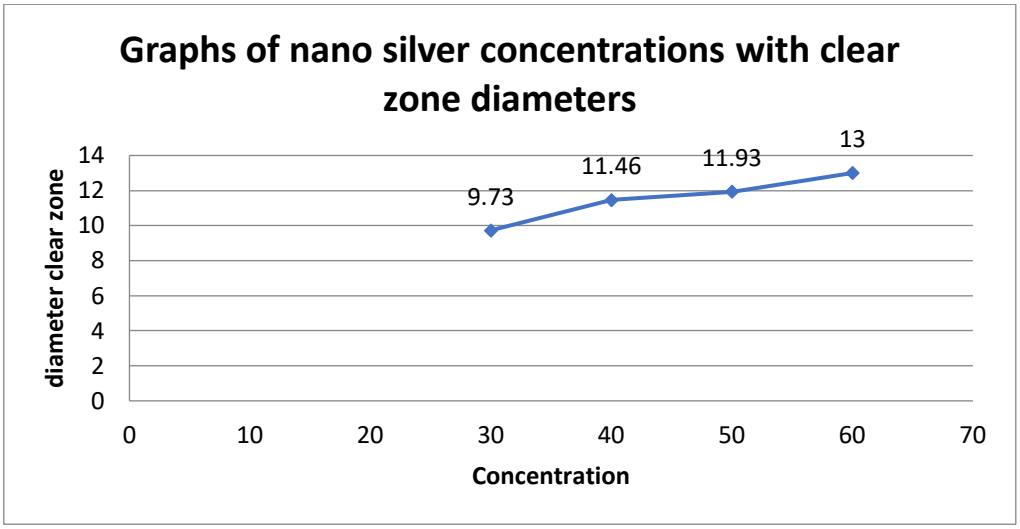

Figure 6: Graphs of nano silver concentrations with clear zone diameters

In this study, four replications were carried out and it can be seen from the results of the diameter of the clear zone that occurs around the disc that the nanosilver in this study has inhibitory properties as an antifungal against Candida albicans. The greater the concentration of nanosilver, the greater the diameter of the clear zone that occurs. In the nanosilver with a concentration of 30 ppm the clear zone diameter that occurred was 9.73 mm, the nanosilver with a concentration of 40 ppm the clear zone diameter that occurred was 11.46 nm, the nanosilver with a concentration of 50 ppm the clear zone diameter that occurred was 11.93 nm, and nanosilver with a concentration of 60 ppm diameter of a clear zone that occurs by 13 mm. According to Mandey and Londok (2014) [22], there are categories of antifungal activity based on inhibition zone diameter that occurs namely <5 mm is weak category, 5-10 mm is moderate category, 10-20 mm is strong category, and >20 is very strong category. Nanosilver in this study included in medium and strong antifungal because at the concentration of 30 ppm the diameter of the inhibitory zone produced was in the range <10 mm or in the category of moderate antifungal and at concentrations of 40, 50, and 60 ppm the diameter of the inhibitory zone produced was included in the range of 10-20 mm or a strong antifungal category against Candida albicans.

In the research of Keuk-jun *et al* (2008) [23], states that the mechanism by which nano-Ag breaks down the membrane permeability barrier, it is possible that nano-Ag perturbs the membrane lipid bilayers, causing the leakage of ions and other materials as well as forming pores and dissipating the electrical potential of the membrane and also the interaction between nano-Ag and the membrane structure. C. albicans cells, during nano-Ag exposure, show significant changes to their membranes, which are recognized by the formation of 'pits' on their surfaces, and finally, the result in the formation of pores and cell death. So nanosilver can inhibit the growth of Candida albicans through destruction of membrane integrity; therefore, it was concluded that nano-Ag has strong antifungal activity against Candida albicans.

The positive control used in this study was ketocenazole which produced clear zone diameter at 16.16 mm. Ketocenazole is also included in a powerful antifungal to inhibit the growth of Candida albicans. While the negative control used in this study is aquadest, in negative control (aquades) no clear zone appears around the disc. The absence of this clear zone indicates that aquades has no antifungal activity and can not inhibit the growth of Candida albicans.

4. CONCLUSION

Based on the results of the study it can be seen that nanosilver in this study does not have antibacterial activity against Neisseria gonorrhoeae and can not inhibit the growth of Neisseria gonorrhoeae as indicated by the absence of clear zone that occur around the discs, but nanosilver in this study has a strong antifungal activity in inhibiting growth Candida albicans, with an increase in concentration on nanosilver can increase the growth inhibition of Candida albicans shown by a wider diameter of the clear zone that occurs around the disk. The clear zone diameters that occur in Candida albicans were at a conmcentration of 30 ppm at 9.73 mm, 40 ppm at 11.46 mm, 50 ppm at 11.93 nm, and 60 ppm at 13 mm.

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None.

CONFLICT OF INTEREST

None.

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