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Antibacterial Activity and the Hydrophobicity of Cotton Coated with Hexadecyltrimethoxysilane

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Abstract. In this work, cotton fiber was fabricated using silver nanoparticles to produce hydrophobic and antibacterial material. The silver nanoparticle was prepared with chemical reduction method using trisodium citrate as reducing agent and PVA as stabilizer. Silver nanoparticle was deposited on cotton fibers as antibacterial agent and HDTMS 4% v/v was coated on those as hydrophobic agent. The cotton fibers before and after modification were characterized its functional groups, contact angles, and antibacterials activities. The functional groups of cottons were determined by using ATR-FTIR, hydrophobic properties of cottons were determined by measuring contact angle, and antibacterial activities of cottons were determined by measuring clear zone. The addition of HDTMS decreased the intensity of absorption bands of functional groups but increased contact angle of cotton cloth. The cotton cloth-silver nanoparticle shows the highest antibacterial properties. The antibacterial activity of cotton cloth without and with modification against *Staphylococcus aureus* ATCC 25923 and *Eschericia coli* 32518 were significantly different.
Key words: antibacterial, contact angle, cotton, HDTMS, and silver nanoparticles.

INTRODUCTION

The self-cleaning textile materials have attracted considerable interest in material chemistry in recent years. Textile material with antibacterial and self-cleaning is particularly popular among patients, nurses, and physicians. However, this material is hardly found [1]. The self-cleaning textile material with hydrophobic properties has been investigated by mimicking the surface of Lotus (*Nelumbo nucifera*) leaves [2]. The Lotus leaf has micro and nano-scale structure coupled with complex structure that provides the hydrophobic properties. Nanoparticles with a three-dimensional surface structure and gelling additive can be applied to the textile material that provides hydrophobic properties, without reducing breathability and comfort of the material. The coating nanoparticles on textile materials such as cotton fiber, polyurethane foam, and polyester can provide antibacterial properties [3, 4]. The antibacterial material can be developed using silver nanoparticles coated onto textile materials [6-9]. The antibacterial activity of nylon, polyester, cotton-coated nanoparticle is strongly influenced by the size, shape, chemical properties, and the surface roughness [5]. The antimicrobial silver particles are influenced by particle size. The smaller of the particle size, the greater the antimicrobial properties [10,11]. The material with cellulose fiber and deposited silver nanoparticels exhibited interesting hydrophilicity properties, hence it can be used as a vessel artificial blood, non-allergenik, and can be sterilized without affecting the characteristics of the material [12-14].

The antibacterial and self-cleaning material can be achieved through developing a hydrophobic structure using nanoparticles effectively. The nanoparticles can kill many types of microbes in a broad spectrum as well as not toxic to pathogenic microbes. Textile materials with hydrophobic properties can be obtained by modification of textile material. The addition of silane-based molecules with low the surface energy and sufficient hydrocarbon chain length is one way to make the hydrophobic textile materials [15]. Furthermore, the antibacterial properties of the material can be developed with coated silver nanoparticles on the material.

Synthesis of silver nanoparticles can be obtained through two approaches, top-down and the bottom-up approach. The nanoparticle synthesis through a top-down approaches starting from the bulk form, then crushing and grinding process is carried out until the nanometer sized materials. A bottom-up approach is chemically process through the reduction process. In this research, silver nanoparticles were carried out by applying the bottom-up approach using

reduction process. Then the textile material was coated with silver nanoparticles and modified by adding HDTMS to produce hydrophobic textile. At the nano-scale, the silver particles have a physical, chemical, and biological properties, including antibacterial activity [16]. Finally, the antibacterial activities was investigated to achieve the fabrication self-cleaning textile.

EXPERIMENTAL

Materials

Cotton cloth was purchased from the fabric store in Yogyakarta. Silver nitrate, trisodium citrate, polyvinyl alcohol (PVA), ethanol, acetone, and hexadecyltrimetoxysilane (HDTMS) were purchased as commercial products and used as they were without any further purification. Nutrient Agar (NA) and Nutrient Broth (NB) were purchased from Oxoid. Nitrogen gas was purchased from PT Samator, Yogyakarta. *Staphylococcus aureus* ATCC 25923 and *Eschericia coli* 32518 were obtained from collection of Faculty of Medicine, Gadjah Mada University.

Preparation of Silver Nanoparticle by Reduction Method

Preparation of silver nanoparticle was performed using 1×10^{-3} M of silver nitrate solution, 10% trisodium citrate solution, and 0.2% PVA solution. PVA solution and silver nitrate solution were added into three neck flask then refluxed until temperature of 90^0C [17]. Trisodium citrate solution was added dropwise. The reflux process was performed under N_2 purging till the solution transformed to yellow, but stirring was stil done until room temperature reached. Then, the colloidal silver nanoparticles were characterized using UV-Vis spectrophotometer.

Application of Silver Nanoparticles on Cotton Fiber (Cotton - Ag)

Cotton fabric was cut to the size of 10 cm x 15 cm and 5 cm x 5 cm. Cotton fabric was washed by soaking in acetone for 30 minutes and then rinsed or soaked in distilled water nonion for 30 minutes and dried using hairdryer. Then, cotton fiber was immersed in colloidal of silver nanoparticle then twisted around using a shaker with a speed of 153 rpm for 24 hours [18] and dried using hairdryer.

Modification of Cotton Fiber Surface with Compound HDTMS (Cotton - HDTMS)

HDTMS was dissolved in ethanol. Then, cotton and the cotton-Ag were immersed into the HDTMS solution. The reacting process between HDTMS and ethanol solution was carried out at room temperature for 60 minutes. Cotton and cotton-Ag which immersed in silane solution were dried at 80°C for 10 minutes followed by curing at 130°C for 60 minutes [19]. Then, cotton fabric before and after modification were analyzed by using FTIR-ATR spectrophotometer, antibacterial activity test , and contact angle test.

The sample which was prepared in this study were cotton cloth (C_0), cotton cloth-nanoparticles silver (C_1), cotton cloth-HDTMS (C_2), and cotton cloth-silver nanoparticle-HDTMS (C_3), and as control positive was nanoparticles silver coated onto Whatmann.

Characterization

The characteristic of silver nanoparticle was performed using UV-Vis spectrophotometer (Shimadzu UV-2400PC series, Japan). An absorbance of silver nitrate solution 1×10^{-3} M and silver nanoparticle, were measured using a reference solution of distilled water. The functional groups of cotton fiber samples before modification, after being coated silver nanoparticles, after being coated with HDTMS, and after being coated with silver nanoparticles and HDTMS were analyzed by using Fourier Transform Infrared - Attenuated Total Reflactance spectrophotometer (Perkin Elmer FTIR-ATR, Japan).

The anti-dirty (hydrophobic) properties of the samples were determined by measuring the water contact angle (θ) between the fluid and the sample surface [20]. Samples were placed on the surface of a table or a flat board and micropipette was placed on the top then paired with the upright. By using a pipette, liquid was dripped from a height

of 1 cm of the sample. Once the liquid dipped, the contact angle shooting was done. The images were processed using software to determine the contact angle between the liquid surface of the sample.

Antibacterial activity was performed by preparing bacterial growth in media such as Nutrient Agar (NA) and Nutrient Broth (NB) by dissolving 14 grams of NA in 500 mL of distilled water and 2 grams of NB in 250 mL distilled water. All the tools and media for growing bacteria were sterilized in autoclave. Rejuvenation of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 32518 were performed on an agar medium NA and incubated for 24 hours at room temperature. *Staphylococcus aureus* 25923 and *Escherichia coli* ATCC 32518 which has been rejuvenated for 24 hours and then inoculated into a liquid medium NB in the culture bottles and incubated for 24 hours at a temperature of 37^0C. Meanwhile, NA was poured into each petri of approximately 10 mL and kept for about 24 hours. Petri dish which had been ascertained so that no contamination was then coated with NB which had been overgrown with bacteria and leveled using drygalski. Each sample was cut to a diameter of 0.5 cm then, inserted into the petri dish and allowed in the incubator for 24 hours, then was observed a clear zone every three hours for 96 hours.
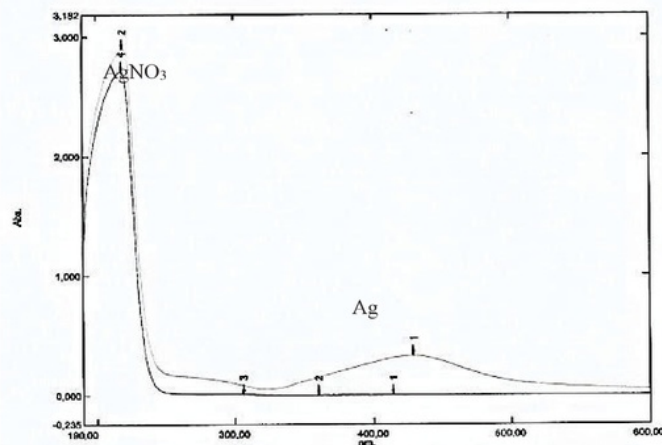
RESULTS AND DISCUSSION

Characteristic of Silver Nanoparticle

Figure 1 shows the UV-Vis spectra of the silver nitrate solution and silver nanoparticles.

FIGURE 1. The Uv-Vis spectra of the silver nitrate solution (a) and silver nanoparticle (b).

Peak at wavelength region 429 nm indicates the formation of Ag^0 Nanoparticles. This is consistent with the works of Barud *et al*. [14], Saputra *et al*. [21], and Ibrahim [22]. According to Table 1, a peak at 3280.68 and 3286.68 cm^{-1} is assigned as vibration-OH group of cellulose (cotton cloth). The next peak found at 2839.88 and 2898.06 cm^{-1} is to be vibration-C-H bond in the -CH$_2$ -CH symmetry and asymmetry, indicating the long hydrocarbon chains of the HDTMS compound [2]. Si-O-Si bonds are at the peak of 1024.38 cm^{-1} and 1025.77 cm^{-1}. Si-O-Si bond is contained in HDTMS compound. Based on the FTIR, Si-OH and Si-C which are usually located at about 800-900 cm^{-1} and 700-800 cm^{-1} are not so obvious. Si-OH bond is formed from the hydrolysis between HDTMS and alcohol.

Functional Groups and Contact Angles of Cotton Cloths

Table 2 shows the highest contact angle on the sample of cotton fabric which was coated HDTMS without silver nanoparticles, i.e. 126.5^0. HDTMS can bind to the -OH of cotton cloth to form Si-OH and provides hydrophobic properties. Additionally, HDTMS has alkoxide group and a long alkyl chain that has the ability to hold water well [23]. The binding between HDTMS compounds with cotton fibers can produce hydrophobic cotton fabrics [24]. The

addition of silver nanoparticles degrades the hydrophobic properties of the fabric. There is a significant differences between the contact angles of pure cotton cloth and a cotton cloth - HDTMS, meanwhile the contact angle of cotton cloth - AgNPs - HDTMS is lower than of pure cotton cloth. These results are consistent with previous studies in that the presences of silver nanoparticles decrease the contact angle.

TABLE 1. Functional groups of cotton before and after modification

Wave number (cm^{-1}) of Sample				Interpretation of Functional Groups
Cotton	Cotton-Ag	Cotton-HDTMS	Cotton-Ag-HDTMS	
3275.87	3276.99	3280.68	3286.68	-OH
2919.58	2917.96	2839.88	2898.05	-H
1728.92	1728	-	-	C=O
1313.63	1313.77	1315.85	1315.22	C-O
1025.11	1026.07	1024.38	1025.77	Si-O-Si
557.87	557.34	593.08	593.14	C-O
510.02	510.17	570.77	552.90	C-O
		525.85	518.08	C-O
		518.11		C-O

TABLE 2. Contact angle of cotton before and after modification

Sample Contact Angle	Cotton	Cotton-Ag	Cotton-HDTMS	Cotton-Ag-HDTMS
Right angle	110^0	104^0	127^0	105^0
Left angle	114	106^0	126^0	108^0
Contact angle (average)	112^0	105^0	126.5^0	106.5^0

Antibacterial Activity of The Cotton Cloth against *Staphylococcus aureus* ATCC 25923 and *Eschericia coli* ATCC 32518

Antibacterial activity test was performed using *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218. The parameter of the analysis is the diameter of the clear zone that appears at around the sample. The clear zone around the sample which is formed by the antibacterial activities of the sample does not grow in this area. The wider clear zone diameter indicates a more effective inhibition against bacteria of the tested sample.

TABLE 3. Antibacterial activity of cotton cloth against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218

Incubation Time (Hours)	Average diameter of the clear zone (mm)							
	Staphylococcus aureus				*Escherichia coli*			
	C$_0$	C$_1$	C$_2$	C$_3$	C$_0$	C$_1$	C$_2$	C$_3$
24	2.167	37.556	4.000	18.667	1.788	28.444	12.111	31.444
48	6.889	44.667	9.833	22.333	8.778	24.556	16.833	27.778
72	22.667	38.778	0.000	21.000	20.333	25.667	12.333	14.667
96	22.889	43.111	0.000	14.167	16.667	22.667	5.333	16.000

Table 3 shows that all test samples have a clear zone in inhibiting of growing bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 at all the incubation time, except samples C$_2$ which show no inhibition zone at 72 and 96 hours. Sample C$_1$ shows the highest antibacterial activity against bacteria *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218. Nevertheless, the lowest antibacterial activity is in the sample C$_2$. Overall, the diameter of inhibition zone on all of the samples tend to decrease in the incubation time of 72 and 96 hours except samples C$_0$. Out of four samples tested, the sample C$_1$ (Cotton - AgNPs) shows the highest clear zone than the other samples.

The silver nanoparticles have a large surface area to facilitate their contact with microorganisms. Silver nanoparticles kill bacteria via the process of diffusion [25]. Research on antibacterial silver nanoparticles were also performed by Patel *et al.* [26] which reported that the antibacterial properties of nanoparticles were associated with its small size, large surface area to make interaction with the higher microbial membrane. Oxygen of -OH in the cotton cloth bound to the silver will bind to sulfihidril (-S-H) on the cell membrane to form a bond R-S-S-R and produce S-Ag clusters that cause lethal inhibition of cell respiration. The cluster S-Ag is very stable on the cell surface of

bacteria because bacteria has sulfhydryl compounds that are not owned by mammals. The silver nanoparticles are not toxic in animals and humans. The AgNO$_3$ deposited on the fabric has antibacterial properties as shown by the structure of chitosan. The chitosan has an amine group to form a positively charged of quaternary ammonium ions to reduce bacterial metabolism through the adsorption. The composition of the chitosan polymer may block the transcription of DNA of the bacteria [27].

   ANOVA test was carried out based on two factors i.e. the incubation time and the type of sample. Table 4 shows that the significance between diameter of clear zone at the different incubation time against the bacteria *Staphylococcus aureus* ATCC 25923 is 0.291 (P > 0, 05), that means there is no significant difference between the antibacterial activity at the different incubation time against the bacteria *Staphylococcus aureus* ATCC 25923. In the test between the types of samples used, the result shows the significance of 0.000 (P < 0, 05) which means that there are significantly the differences in antibacterial activity between the types of samples against *Staphylococcus aureus* ATCC 25923. However, all the difference in the diameter of clear zone at different incubation times and different sample types in inhibiting the growth of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 show a value of 0.000, it means that there are significant differences in terms of antibacterial activity at different incubation times and different sample types.

TABLE 4. Two way ANOVA (two factor) type sample and incubation time against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.	Conclusion
Staphylococcus aureus ATCC 25923						
Time	0.147	19	0.008	1.155	0.291	Not significant
Sample	13.743	3	4.581	682.629	0.000	Significant
Time & Sample	1.464	57	0.026	3.828	0.000	Significant
Escherichia coli ATCC 35218						
Time	0.616	19	0.032	5.699	0.000	Significant
Sample	4.782	3	1.594	280.026	0.000	Significant
Time & Sample	0.630	57	0.011	1.942	0.000	Significant

   Furthermore, the test results LSD (Least Significant Different) between antibacterial activity (clear zone) of the different sample types against *Staphylococcus aureus ATCC* 25923 and *Escherichia coli* ATCC 35218 shows that the diameter of the clear zone between two samples can be significantly different. This means that between the two samples there is different antibacterial activity. The significant results are applicable to all types of samples except for C_0 and C_2 samples which show no significant difference in antibacterial activity against *Escherichia coli* ATCC 35218. Thus, the addition of HDTMS does not affect the antibacterial activity of cotton cloth in inhibiting of growing *Escherichia coli* ATCC 35218 (Table 5).

   The LSD test was also carried out between antibacterial activity of the different incubation time against *Staphylococcus aureus ATCC* 25923 and *Escherichia coli* ATCC 35218. The result shows that the significance between samples are to be > 0.05 and the diameter of the clear zone between the two of incubation time is not significantly different. The non-significant results apply to all comparison of incubation time. This means incubation time does not affect the antibacterial activity. The Clear zone was observed on the first measurement after 24 hours of incubation. Based on thework of Kim *et al.* [28], the antibacterial activity begins at 6 hours of incubation.

TABLE 5. Interpretation of the result test LSD: between diameter of clear zone of samples type against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218

Variable (Type of Samples)	Conclusion	
	Staphylococcus aureus ATCC 25923	*Escherichia coli* ATCC 35218
$C_0 - C_1$	Significant	Significant
$C_0 - C_2$	Significant	Not significant
$C_0 - C_3$	Significant	Significant
$C_1 - C_2$	Significant	Significant
$C_1 - C_3$	Significant	Significant
$C_2 - C_3$	Significant	Significant

The results show that the samples deposited with silver nanoparticles have the highest antibacterial activity. The silver nanoparticles are very likely attached to bacterial cell membrane. Silver nanoparticles react with sulfur protein and phosphorus-containing DNA in a bacterial cell. The reaction causes morphological changes in bacterial cells, DNA damage and respiratory problem, so the bacteria die [10]. The silver nanoparticles were found to accumulate in the bacterial membrane, the bacteria cells were damaged, showing formation of "pits" in the cell wall of the bacteria. A membrane with such morphology exhibits a significant increase in permeability, causing death of the cell [29]. The interaction among nanoAg, CTMAB, and fsDNA through electrostatic and chemical affinity, and the nanoAg–CMAB complex can induce the structural change of base stacking and helicity of fsDNA [30]. The antibacterial properties of silver nanoparticles arising from the electrostatic interaction between silver with negatively-charged bacteria cell surfaces. The Electrostatic interaction between nanoparticles and bacteria was believed to contribute the nanoparticle binding to cell membranes, membrane interference, and reduced cell viability. The positively-charged nanoparticles interacted with negatively-charged bacteria cell surfaces to promote flocculation [31].

The difference antibacterial activity of sample against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 is presented in Figure 2. Based on the figure, the pattern diameter of clear zone against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 of the sample C_0 and C_1 is similar in each measurement. The diameter of clear zone against bacteria *S.aureus* is higher than that against *E.coli*, this means *S.aureus* is more inhibited bacterial growth of the *E.coli*. The statistical tests were performed in this comparison is the t-test with a standard error of 5%. Based on t test, the result shows the significance between *S. aureus* and *E.coli* were 0.206 ($p > 0.05$) for sample C_0 and 0.570 ($p > 0.05$) for sample C_1. Hence it can be concluded that there is no difference between the diameter of the clear zone against *S. aureus* and *E.coli* for sample C_0 (cotton cloth) and sample C_1 (cotton cloth - silver nanoparticle).

In this study, all samples have antibacterial activity against *S. aureus* and *E. coli*. The cotton cloth coated with silver nanoparticles shows anti-bacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Textiles coated silver nanoparticle shows antibacterial activity against *Escherichia coli* and *S. aureus* [1, 3, 4-6, 8-9], *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus mirabilis*, *K. pneumoniae*, *Candida albicans* yeasts, and micromycetes [32]. Presumably, under the impregnation of cotton with metal ions and ironing at 200°C, the basis of cotton, cellulose, simultaneously reduces of ions and stabilizes the appearing nanoparticles [32]. Cellulose is a long chain polymer molecule consisting of repeating glucosidic residues, 300–10,000 glucose residues, without side loops. Cellulose contains reducing oligosaccharides. Their aldehyde function presumably can promote the process of silver ion reduction (probably analogous to the reaction of "silver mirror ") [32].

The silver nanoparticles deposited onto cotton samples exhibit quite high the diameter of clear zone. As mentioned above, this is because the silver nanoparticles have antibacterial properties. Silver can attack biological processes in microorganisms including cell membrane structure and functions. Silver also inhibits the expression of proteins associated with ATP production [33]. Figure 2 provides the information that the diameter of the clear zone on *S. aureus* is higher than on *E.coli* (for Sample C_0 and C_1). It indicates that *S. aureus* is more obstructed than *E.coli*. *S. aureus* (gram positive) which only has a single plasma membrane surrounded by thick walls consisting of 90 % of peptidoglycan teikhoic acid [29]. Meanwhile, *E. coli* (gram negative) has the double membrane system. The plasma of *E. coli* is enveloped by outer membrane permeable and surrounded by a thick wall of peptidoglycan which between the outer membrane and the inner membrane. This makes silver nanoparticles easier inhibit the growth of *S. aureus* (gram-positive bacteria) than *E.coli* (gram negative bacteria).

Figure 2 also provides that the diameter of the clear zone of samples C_2 and C_3 against *E. coli* is higher than that against *S.aureus*. This indicates that the growth of *E.coli* is more inhibited than *S.aureus*. The t test of bacteria shows significance of 0.000 ($p < 0.05$) for sample C_2 and significance of 0.054 ($p > 0.05$) for sample C_3. Therefore, it is found a significant difference in diameter of the clear zone between *S.aures* and *E. coli* in a sample C_2 (Cotton - HDTMS) but no difference between the diameter of the clear zone on *S. aureus* and *E.coli* in the sample C_3 (Cotton - AgNPs - HDTMS). The HDTMS compounds can increase the contact angle (Table 2), to change the surface of the cotton fabric becomes more hydrophobic. The presence of hydrophobic surfaces can kill cells of *E. coli* bacteria.
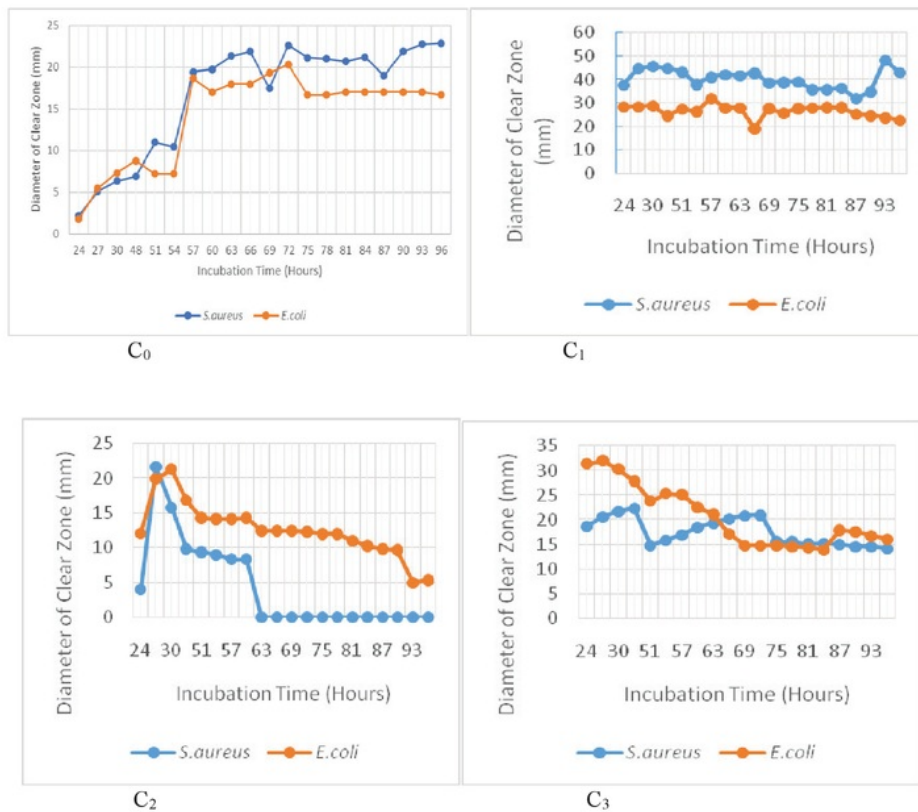
FIGURE 2. The diameter of clear zone of cotton cloth against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 35218 on C_0, C_1, C_2 and C_3.

The surface of cellulose derivatives which are coated with different quarternary ammonium groups and additional hydrophobic groups [34] can kill *S. aureus* cells, and this is mainly controlled by hydrophobic balance and not by the density of charge. The antimicrobial surface can be obtained by coating the surfaces using the silane 3-(trimethoxysilyl) propyldimethyloctadecylammonium chloride [35]. The presence of tertiary amino groups which can be protonated at random copolymer of styrene and octylstyrene dimethylaminomethyl causes the compound to have antibacterial activity [36]. The copolymerization between dimethylaminoethylacrylamide and aminoethylacrylamide with *n*-butylacrylamide shows antibacterial properties and less toxic to cells than the quaternary ammonium derivatives [37, 38]. The poly(allylammonium) salt containing an amino group either secondary or tertiary [3] exhibits excellent antibacterial activity against *S. aureus* and *Candida albicans*. In addition to linear polymers, dendritic, and hyperbranched polymers can also have strong antimicrobial properties [40]. Thus, the addition of HDTMS can improve more hydrophobic surfaces causing extremely difficult to wet [2], and can enhance the antibacterial activity of cotton cloth in inhibiting the growth of bacteria *E.coli*. However, cotton cloth deposited with silver nanoparticles shows the highest antibacterial activity, followed by cotton cloth coated with silver nanoparticles and HDTMS, then pure cotton cloth, and the lowest is cotton cloth coated HDTMS.

CONCLUSION

Silver nanoparticles can be prepared from a solution of silver nitrate with a reductant of tri-sodium citrate, and formed at a wavelength of 429 nm. The addition of HDTMS decreases the absorption intensity of functional groups but increases the contact angle of cotton cloth. The cotton cloth-HDTMS has the highest contact angle. There is a significant difference in antibacterial activity among all samples. The cotton cloth-nanoparticle silver has the highest

antibacterial properties. The antibacterial activity of cotton cloth without and with modification against *Staphylococcus aureus* ATCC 25923 and *Eschericia coli* 32518 are significantly different.

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