Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity

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Background: There is worldwide interest in silver nanoparticles (AgNPs) synthesized by various chemical reactions for use in applications exploiting their antibacterial activity, even though these processes exhibit a broad range of toxicity in vertebrates and invertebrates alike. To avoid the chemical toxicity, biosynthesis (green synthesis) of metal nanoparticles is proposed as a cost-effective and environmental friendly alternative. Aloe vera leaf extract is a medicinal agent with multiple properties including an antibacterial effect. Moreover the constituents of aloe vera leaves include lignin, hemicellulose, and pectins which can be used in the reduction of silver ions to produce as AgNPs@aloe vera (AgNPs@AV) with antibacterial activity. **Methods:** AgNPs were prepared by an eco-friendly hydrothermal method using an aloe vera plant extract solution as both a reducing and stabilizing agent. AgNPs@AV were characterized using XRD and SEM. Additionally, an agar well diffusion method was used to screen for antimicrobial activity. MIC and MBC were used to correlate the concentration of AgNPs@AV its bactericidal effect. SEM was used to investigate bacterial inactivation. Then the toxicity with human cells was investigated using an MTT assay. **Results:** The synthesized AgNPs were crystalline with sizes of 70.70 ± 22-192.02 ± 53 nm as revealed using XRD and SEM. The sizes of AgNPs can be varied through alteration of times and temperatures used in their synthesis. These AgNPs were investigated for potential use as an antibacterial agent to inhibit pathogenic bacteria. Their antibacterial activity was tested on *S. epidermidis* and *P. aeruginosa*. The results showed that AgNPs had a high antibacterial which depended on their synthesis conditions, particularly when processed at 100 °C for 6 h and 200 °C for 12 h. The cytotoxicity of AgNPs was determined using human PBMCs revealing no obvious cytotoxicity. These

results indicated that AgNPs@AV can be effectively utilized in pharmaceutical, biotechnological and biomedical applications. **Discussion:** Aloe vera extract was processed using a green and facile method. This was a hydrothermal method to reduce silver nitrate to AgNPs@AV. Varying the hydrothermal temperature provided the fine spherical shaped nanoparticles. The size of the nanomaterial was affected by its thermal preparation. The particle size of AgNPs could be tuned by varying both time and temperature. A process using a pure AG phase could go to completion in 6h at 200 °C, whereas reactions at lower temperatures required longer times. Moreover, the antibacterial effect of this hybrid nanomaterial was sufficient that it could be used to inhibit pathogenic bacteria since silver release was dependent upon its particle size. The high activity of the largest AgNPs might have resulted from a high concentration of aloe vera compounds incorporated into the AgNPs during hydrothermal synthesis.

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Abstract

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Results: The synthesized AgNPs were crystalline with sizes of 70.70 ± 22-192.02 ± 53 nm as revealed using XRD and SEM. The sizes of AgNPs can be varied through alteration of times and temperatures used in their synthesis. These AgNPs were investigated for potential use as an antibacterial agent to inhibit pathogenic bacteria. Their antibacterial activity was tested on *S. epidermidis* and *P. aeruginosa*. The results showed that AgNPs had a high antibacterial which depended on their synthesis conditions, particularly when processed at 100 ^{o}C for 6 h and 200 ^{o}C for 12 h. The cytotoxicity of AgNPs was determined using human PBMCs revealing no obvious

cytotoxicity. These results indicated that AgNPs@AV can be effectively utilized in pharmaceutical, biotechnological and biomedical applications.

Discussion: Aloe vera extract was processed using a green and facile method. This was a hydrothermal method to reduce silver nitrate to AgNPs@AV. Varying the hydrothermal temperature provided the fine spherical shaped nanoparticles. The size of the nanomaterial was affected by its thermal preparation. The particle size of AgNPs could be tuned by varying both time and temperature. A process using a pure AG phase could go to completion in 6 h at 200 ^{o}C, whereas reactions at lower temperatures required longer times. Moreover, the antibacterial effect of this hybrid nanomaterial was sufficient that it could be used to inhibit pathogenic bacteria since silver release was dependent upon its particle size. The high activity of the largest AgNPs might have resulted from a high concentration of aloe vera compounds incorporated into the AgNPs during hydrothermal synthesis.

Introduction

Silver nanoparticles (AgNPs) have been extensively studied for many decades due to their unique features and wide range of applications. Their uses include catalysis *(Pradhan, Pal & Pal, 2002)*, biosensing *(Anker et al., 2008)*, imaging *(Lee & El-Sayed, 2006)*, and antibacterial activity *(Morones et al., 2005; Rai, Yadav & Gade, 2009)*. Among these applications, antibacterial activities have gained much attention because they potentially offer a solution to the problem of antibiotic resistance *(Cho et al., 2005)*. There are a variety of methods to synthesize AgNPs including physical and chemical methods *(Chudasama et al., 2010)*. Chemical reduction of silver ions using sodium borohydride *(Zhang et al., 2000)*, hydrazine *(Taleb, Petit & Pileni, 1997)*, ascorbic acid *(Lee et al., 2004)*, trisodium citrate *(Sun Mayers & Xia, 2003)*, and polyols *(Sun &*

Xia, 2002) were reported and are considered well-established methods. Although chemical routes are effective, these methods may suffer from toxicity due to the chemicals used and the difficulty in removing them. Additionally, chemical reagents used in these methods are hazardous to the environment *(Nabikhan et al., 2010)*. To avoid the toxicity of chemicals, green synthesis was developed *(Sharma, Yngard & Lin, 2009)*. This method of biosynthesis of metal nanoparticles has been proposed as a cost-effective and environmental friendly way of fabricating these materials.

Synthesis of AgNPs employing either microorganisms or plant extracts has emerged as an alternative approach. These biosynthetic methods have a numbers of benefits. They are simple, cost-effective, give high yields, and are environmentally friendly *(Zhang et al., 2013)*. Plant extracts have reportedly been used in the preparation of AgNPs *(Sun et al., 2014)*. Aloe vera leaves have been used as medicinal plants since they possess anti-inflammatory activity, UV protection, anti-arthritic properties, promote wound and burn-healing, and have antibacterial properties *(Chandran et al., 2006; Feng et al., 2000; Reynolds & Dweck, 1999; Vazquez et al., 1996)*. There are a number of biologically active constituents in aloe vera leaves. These include lignin, hemicellulose, pectins which can be used in the reduction of silver ions *(Emaga et al., 2008)*. It is believed that the large enzymes and proteins in aloe vera extract are weakly bound to silver ions and function as a complexing agent. Due to their low cost and environmentally friendly nature coupled with their reducing properties, we selected aloe vera as the reducing and stabilizing agent to prepare AgNPs and test their antibacterial activity.

In this study, we report a one-step hydrothermal method to prepare silver nanoparticles. Reduction of Ag^{+} ions to Ag^{0} nanoparticles was done in a medium of aloe vera extract in which no extra reducing agent was used. The resulting AgNPs can be obtained in large quantities. The sizes of AgNPs were found to be in a range of 70.70-192.02 nm and controllable by varying temperature

95 and time conditions of the hydrothermal process. Further, the resulting AgNPs were found to be
96 effective against gram-positive (*Streptococcus epidermidis*) and gram-negative (*Pseudomonas*
97 *aeruginosa*). Therefore, this work has shown the use of naturally occurring compounds to be a
98 reducing and stabilizing agent. This method is considered green synthesis. The resulting silver
99 nanoparticles showed a synergism of aloe verla and silver nanoparticles on bactericidal effect. This
100 hybrid nanomaterial provides an alternative material for using in antibacterials.
101
102 **Materials and Methods**
103 In this study, silver nitrate, $AgNO_3$ (Sigma-Aldrich Chemicals, USA) and aloe vera plant extract
104 were used as the starting materials. The aloe vera extract solution was prepared using 50 g of aloe
105 vera leaves that had been rinsed with deionized water and finely cut into small pieces. The chopped
106 aloe vera leaves were boiled in a 50 mL of deionized water for 20 minutes and allowed to cool.
107 The cooled leaf broth was filtered and stored in a refrigerator at 4 °C. The resulting extract was
108 used as an aloe vera extract solution.

109 **Synthesis of AgNPs and Characterization of AgNPs**

110 In the preparation of AgNPs samples, $AgNO_3$ (0.3 mol) was first dissolved in 20 ml of deionized
111 water and mixed with 20 ml of aloe vera extract solution under vigorous stirring at room
112 temperature for 30 minutes. The mixtures were added to sealed Teflon-lined vessels of 100 mL
113 capacity (Parr, USA), which were heated and maintained at various time and temperature
114 conditions, and then gradually cooled to room temperature. A gray precipitate was collected by
115 filtration and washed with deionized water several times, and finally dried in air at 60 ^{o}C for 6 h.
116 The crystal phase analysis of the AgNPs powders was conducted using X-ray diffraction (XRD)
117 (PW3710, the Netherlands) with CuKα radiation ($\lambda = 0.15406$ nm). The particle sizes and

morphology of the prepared AgNPs samples were characterized using scanning electron microscopy (SEM) (LEO SEM 1450VP, UK) and transmission electron microscopy (TEM) (FEI 5022/22 Tecnai G2 20 S-Twin, CR). The UV–visible absorbance of the AgNPs was measured using UV-1800 (Shimadzu, Japan).

Antibacterial Tests and Cytotoxicity Test

Well diffusion method

The antibacterial activity of AgNPs prepared under different hydrothermal processing conditions were tested against gram-negative *P. aeruginosa* (*Pseudomonas aeruginosa*, ATCC27803) and gram-positive *S. epidermidis* (*Staphylococcus epidermidis*, ATCC35984) using an agar well diffusion method. The organisms were sub-cultured in nutrient broth at 37 ^{o}C and incubated overnight. After that, Nutrient Agar (Merck) was swabbed with the respective sub-cultures (1×10^{8} CFU/ml). Specimens containing AgNPs were then arranged on the swabbed agar surface and incubated at 37 °C for 24 h. The results were read by measuring the diameter of the inhibition zone (mm). The experiments were done in triplicate.

Scanning electron microscopy (SEM)

Scanning electron microscopy of control cells and AgNPs treated cells (0.04 mg/mL) was performed to investigate the antibacterial activity. Each bacterial culture was prepared as described above and then pipetted into a 6-well plate with and without AgNPs prior to covering the wells with glass slides. After incubating at 37 °C overnight, the glass slides were removed and gently washed with phosphate buffer saline 3 times before dehydration in an alcohol series using concentrations of 25%, 50%, 75%, 90% and 100% ethanol in distilled water. The slides were left

in each concentration for 20 minutes. They were then air dried and kept in a desiccator until analysis.

Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

A microdilution method was used to indicate the bactericidal effect of AgNPs. A suspension of 1×10^8 CFU/ml of bacteria in nutrient broth was prepared as described above. The antibacterial solutions were prepared using serial two-fold (1:2) dilutions of AgNPs in concentrations ranging from 0.04 to 0.00008 mg/mL and incubated at 37 ^oC for 24 h. In the range of sample turbidity, the MIC of the samples could not be determined to identify the lowest concentration of antibacterial agent that inhibits 99% of the growth of the bacteria. A microdilution measurement was done in triplicate to confirm the value of MIC for each tested bacteria. As such, the MBC was measured after MIC determination. In this assay, 10 µl from all concentrations of AgNPs were pipetted onto nutrient agar plates and incubated at 37 ^oC for 24 h. The MBC endpoint was interpreted at the lowest concentration of antibacterial agent killing 100% of the initial bacterial population.

Cytotoxicity Test

The AgNP samples produced at100 ^oC for 6 h and 200 ^oC for 12 h were tested for their cytotoxicity using the MTT3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Human peripheral blood mononuclear cells (PBMCs) from the leftover buffy coat were suspended into complete 1640 RPMI (supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 unit/ml penicillin and 100 µg/ml streptomycin) in a 96-well plate at a density of 10^5 cells/well. This was done prior to exposure to AgNPs dissolved in RPMI to make a stock concentration at 0.04 mg/mL. The stock solution was used to generate serial two-fold dilution at 4 concentrations, i.e., 0.02, 0.01, 0.005, and 0.0025 mg/mL. Then, the cells were incubated at 37 ^oC in a fully humidified, 5% CO_2 air atmosphere for 48 h. The test samples were removed from the cell cultures

and the cells were reincubated for a further 24 h in fresh medium. They were then tested using the MTT assay. Briefly, 50 µl of MTT in phosphate buffered saline at 5 mg/ml was added into a medium in each well and the cells were incubated for 4 h. The medium and MTT were then gently aspirated from the wells and solubilized in formazan with 200 µl of DMSO and 25 µl of Sorensen's Glycine buffer, pH 10.5. The optical density was read with a microplate reader at a wavelength of 560 nm. The average of 3 wells was used to determine the mean of each point. Then % survival of the cells was calculated. For each test sample, the data was used to determine the concentration of sample required to kill 50% (IC_{50}) of the cells compared to that of the controls. A dose-response curve was derived from 5 concentrations in the test range using 3 wells per concentration.

Results

Characterization of Silver Nanoparticles

The morphology of AgNPs prepared at different reaction temperatures and times was examined using SEM. The result showed SEM images of AgNPs obtained by the reduction of $AgNO_3$ with aloe vera plant extract (Fig. 1). It was found that the reaction time and temperature had significant effects on the formation of Ag nanostructures. AgNPs were observed as spherical particles with the sizes between 70.7-192.02 nm, moreover the sizes of the materials were significantly affected by their preparation temperature as presented in Table 1. At 6 h, the AgNPs showed the sizes of 70.70 ± 23, 79.47 ± 22, and 161.66 ± 53 nm prepared at 100 ^oC, 150 ^oC and 200 ^oC, respectively. At 12 h, the AgNPs showed sizes of 95.25 ± 23, 149.55 ± 47 and 192.02 ± 53 nm prepared at 100 ^oC, 150 ^oC and 200 ^oC, respectively. Furthermore, TEM image of AgNPs prepared at 100 ^oC for 6 h indicating that the size of AgNPs was in good agreement with SEM results, UV-vis absorption spectra of AgNPs showed that the maximum absorption was found at 420 nm and was attributed

186 to the surface plasmon resonance of AgNPs (Figs. 2A and 2B). The XRD patterns of AgNPs
187 resulted from using the above 3 hydrothermal conditions (Figs. 3A and 3B). All of the main peaks
188 were indexed as AgNPs with the face centered cubic (fcc) lattice of silver, as shown in the standard
189 data (JCPDS file No.01-071-4613). The diffraction peaks at 2θ degree of 38.2, 44.3, 64.5 and 77.1
190 corresponded to the (111), (200), (220), and (311) planes, respectively. A pure phase of Ag was
191 only obtained at a temperature of 200 ^{o}C for 6 h. The chemical reaction to form a pure phase at
192 100 and 150 ^{o}C for 6 h was incomplete because reaction at such a low temperature usually requires
193 a longer time (Fig. 3A). The existence of Ag_2O was shown at the peak at around 31.9 *(Liu et al.,*
194 *2010)*. The result showed a pure Ag phase in all the samples prepared using hydrothermal
195 conditions for 12 h (Fig. 3B).

196 **Antibacterial Effects**

197 An advantage of silver nanoparticles is that they are known to have an antibacterial effect *(Rai et*
198 *al., 2012)*. However, the AgNPs formed during the aloe vera hydrothermal method, AgNPs@AV,
199 need to have bioactive functions. It is especially important to understand the functional effects on
200 microorganisms in order to develop novel antibacterial agents. To demonstrate this activity,
201 AgNPs were studied for their bactericidal effect against pathogenic gram-positive *S. epidermidis*
202 and gram-negative *P. aeruginosa*. These two stains are the opportunistic bacteria causing of
203 nosocomial infection, moreover there are the virulent factors involving with antibiotic resistance
204 *(Otto 2009; Livermore 2002)*. Thus, our new product might be a material of choice to apply in
205 antimicrobial activity instead of antibiotics. This was done using a qualitative antibacterial well
206 diffusion assay and studying AgNPs interaction with bacteria using SEM. Quantitative
207 antibacterial concentrations were evaluated by determining the minimum bactericidal
208 concentration (MBC). It was observed that the inhibition zones of both pathogens were significant

for 0.1 mg/mL AgNPs prepared at 100 °C for 6 h, 150 °C and 200 °C for 12 h compared with the control (Fig. 4 and Table 1). The AgNPs synthesized under different conditions provided varying bactericidal effects. Then, the effects of two AgNPs@AV samples including those prepared at 100 °C for 6 h and 200 °C for 12 h, were selected for further studies using SEM and MBC. The interaction of AgNPs and microorganisms was shown using SEM. The result indicated the cell membrane changed when contacted with the nanoparticles (Fig. 5). This was particularly true for gram-negative bacteria, showing a thin layer of membrane and having pores. Subsequently, MBC of the bacterial concentration at 10^8 CFU/ml was determined for both *S. epidermidis* and *P. aeruginosa*. This demonstrated the lowest concentration of nanoparticles with bactericidal effect was 0.01 mg/mL for AgNPs fabricated at 100 °C for 6 h and 200 °C for 12 h against 10^8 CFU/ml *S. epidermidis*. The corresponding concentrations was 0.0025 mg/mL for AgNPs fabricated at 100 °C for 6 h and 0.00125 mg/mL for those formed at 200 °C for 12 h against 10^8 CFU/ml *P. aeruginosa*. Moreover, the microbicidal activity of nanoparticles provided high efficiency within 2 months. At the lower AgNPs concentrations, clearly there was an effect on the lethality against gram negative-bacteria whereas higher concentrations were needed to control gram-positive bacteria.

Cytotoxicity Evaluation

To determine the cytotoxicity of AgNPs@AV on human cells, PBMCs were tested using the MTT assay. The result was calculated as %survival of the cells cultured with samples at concentrations of 0.04, 0.02, 0.01, 0.005, and 0.0025 mg/mL of 100 °C for 6 h and 200 °C for 12 h processed AgNPs@AV. The %survival of the cells in less 0.0025 mg/mL of both nanoparticles was significantly higher than 50% which confirms that these AgNPs@AVs were non-toxic to human PBMCs. Nanoparticles produced by green synthesis can be useful in biomedical applications.

232

233 **Discussion**

234 Recently, there has been increasing study of AgNPs synthesis to develop several applications such
235 as catalysis, biosensing, imaging, and antibacterial activity. Green synthesis is an alternative
236 method developed to produce metal nanoparticles by using natural compounds or plant
237 components. These are environmentally friendly processes that avoid the toxicity of chemicals.
238 Algae, bacteria, fungi and plants have been used to synthesize NPs without the need for additional
239 reducing and stabilizing agents. Plant extracts contain functional substances, including cyclic
240 peptides, sorbic acid, citric acid, euphol, polyhydroxy limonoids, ascorbic acid, retinoic acid,
241 tannins, ellagic acid, and gallic acid, among others, are strongly believed to play a crucial role in
242 the bioreduction and stabilization of nanoparticles *(Rajan et al., 2015)*. These processes seem
243 facile, safe, low cost, and ecofriendly, eliminating the elaborate process of maintaining aseptic cell
244 cultures and are suitable for large scale production. Therefore, this study focused on the
245 biosynthesis of AgNPs with plant extracts of aloe vera leaves. Zhang et al. (*2010*) speculated that
246 the hydroquinones in the aloe vera plant extract act as the reducing agents. Additionally, the
247 spherical shape of AgNPs was governed by the weaker binding of proteins in the solution leading
248 to the isotropic growth of the AgNPs. Here, the hydrothermal process was applied to AgNPs
249 synthesis in which time and temperature had an effect on the resulting crystalline structure of
250 AgNPs. High temperature and pressure are necessary to facilitate the reduction processes *(Liu et*
251 *al., 2012)*. Nucleation and the growth of AgNPs depend on the reaction temperature. Additionally,
252 capping agents also play a role in the synthesis of nanoparticles. Selective interaction of capping
253 agents may lead to anisotropic crystalline growth. Poly (vinyl) pyrrolidoneis are widely used to
254 synthesize nanorods due to their preferential interaction with the (100) plane *(Pal, Tak & Song,*

2007). In the case of aloe vera, a (111) plane of AgNPs predominantly arose as a major peak. This plane was reported responsible for a strong antibacterial effect *(Feng et al., 2000)*.

The factors controlling the morphology, size, and product purity in the hydrothermal process were reaction temperature and time *(Byrappa & Adschiri, 2007; Liu et al., 2014)*. Moreover, biosynthesis of inorganic nanoparticles with the plant extracts improved their bactericidal effect *(Yousefzadi, Rahimi & Ghafori, 2014)*. High bactericidal activity was possibly caused by synergistic antibacterial effects of AgNPs and naturally-occurring chemicals in aloe vera. The lethal mechanism against pathogenic *S. epidermidis* and *P. aeruginosa* might involve the release of Ag^+ ions from AgNPs and the formation of crystalline bio-organic compounds of aloe vera plant extract assembled with AgNPs anchored onto the bacterial cell walls, producing pits and penetrating into the cytoplasm. Various natural ligands can interact with microbial membrane such as saponin, tannin, terpenoids, and flavonoids in the aloe vera *(Griffin et al., 1999; Sahu et al., 2013)*. The interaction with the cell membrane may increase its permeability leading to cell lysis. Moreover the free radicals from metal result in induction of oxidative stresses, such as reactive oxygen species (ROS), that can damage the bacterial membranes, mitochondria, and DNA. This eventually results in the death of the cell *(Hajipour et al., 2012; Tamboli & Lee, 2014)*. From our results, a schematic mechanism involving the reaction of AgNPs@AV to kill the bacteria was purposed and illustrated in Figure 6. Additionally, the susceptibility of different types of bacteria was attributed to the structure of their bacterial cell walls. Previous studies indicated that the silver ion released from AgNPs was responsible for antibacterial activity *(Feng et al., 2000)*. The free silver ion can then bind with the thiol groups of enzymes *(Zhang et al., 2013)*. The AgNPs formed at 100 ^oC for 6 h were found to be toxic to both gram-positive and gram-negative bacteria. This might due to the smaller size of the AgNPs fabricated under these conditions which results a higher

surface area *(Cui et al., 2013)*. Silver ion release is a size dependent process *(Cui et al., 2013)*. The antibacterial activity of the synthesized AgNPs might be due to the silver ion release and the resulting genotoxic activity of aloe vera on *E. coli (Zhang et al., 2010)*. Interestingly, the samples processed at 200 ^{o}C for 12 h had the largest size of those examined and they provided effective growth inhibition of the pathogens. The results indicated that the larger AgNPs might contain high levels of incorporated aloe vera compounds as well as a pure Ag phase due their long time and high temperature treatment. Therefore, this hybrid nanostructure formed under specific conditions can potential be an antibacterial agent.

Conclusion

This report described a green and facile method to synthesize AgNPs in large quantities. Silver nitrate was reduced in an aloe vera plant-extract solution under a hydrothermal condition. Aloe vera plant extract solutions were used as both reducing and stabilizing agents. Fine spherically shape nanoparticles were obtained. The particle size of AgNPs can be tuned by varying the hydrothermal temperature. The antibacterial effect of AgNPs@AV showed promise for use as a highly potent agent with minimal cytotoxicity to human PBMCs. These hybrid nanomaterials could potentially be used in biomedical applications.

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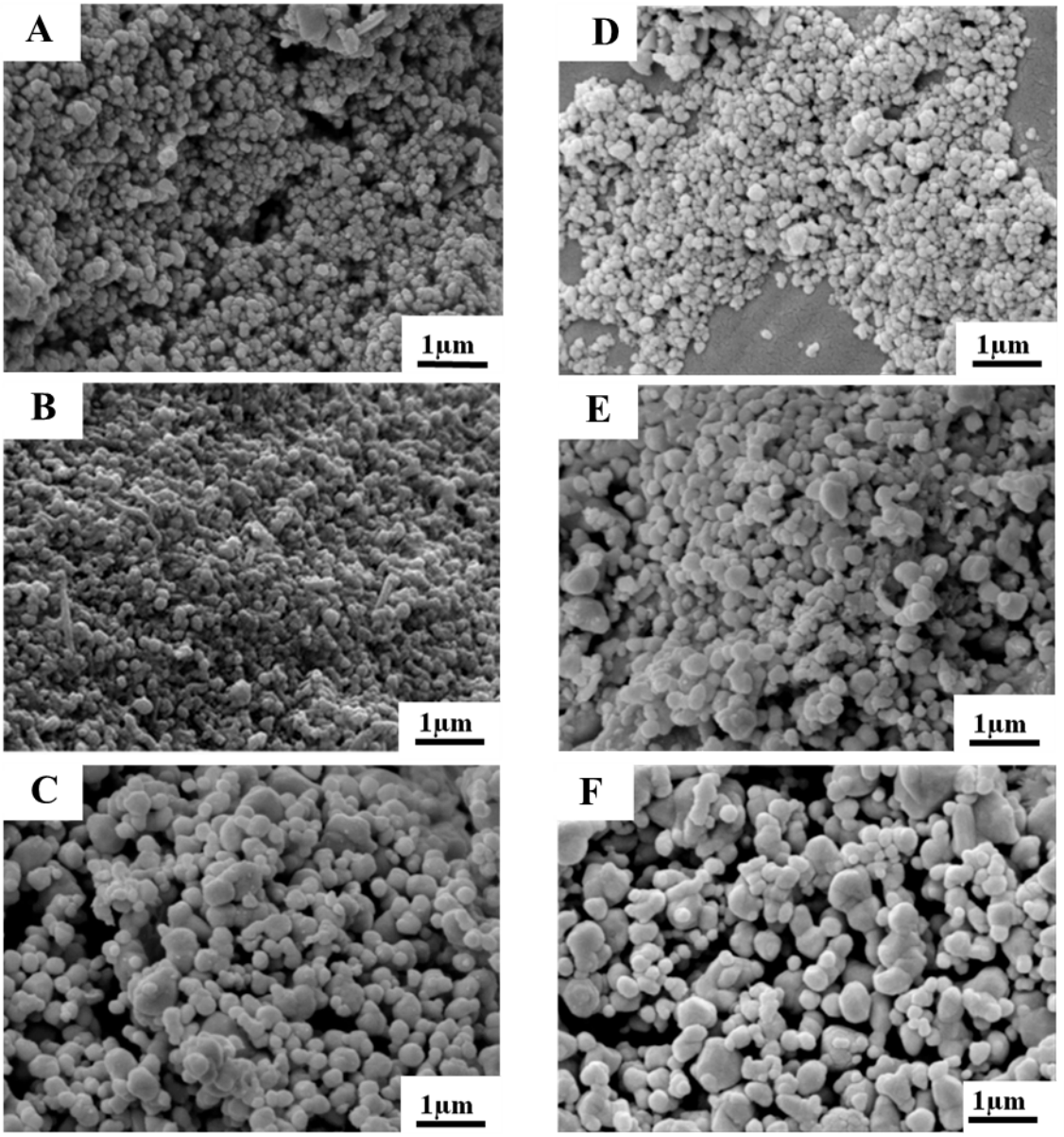
398 **LIST OF FIGURES AND LEGENDS**



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400 **Figure 1 SEM images of silver nanoparticles on a glass slide after incubation at different**

401 **temperature and time combinations.** SEM images of AgNPs were obtained at (A) 100 °C for

402 6 h, (B) 150 °C for 6 h, (C) 200 °C for 6 h, (D) 100 °C for 12 h, (E) 150 °C for 12 h and (F) 200

403 °C for 12 h.

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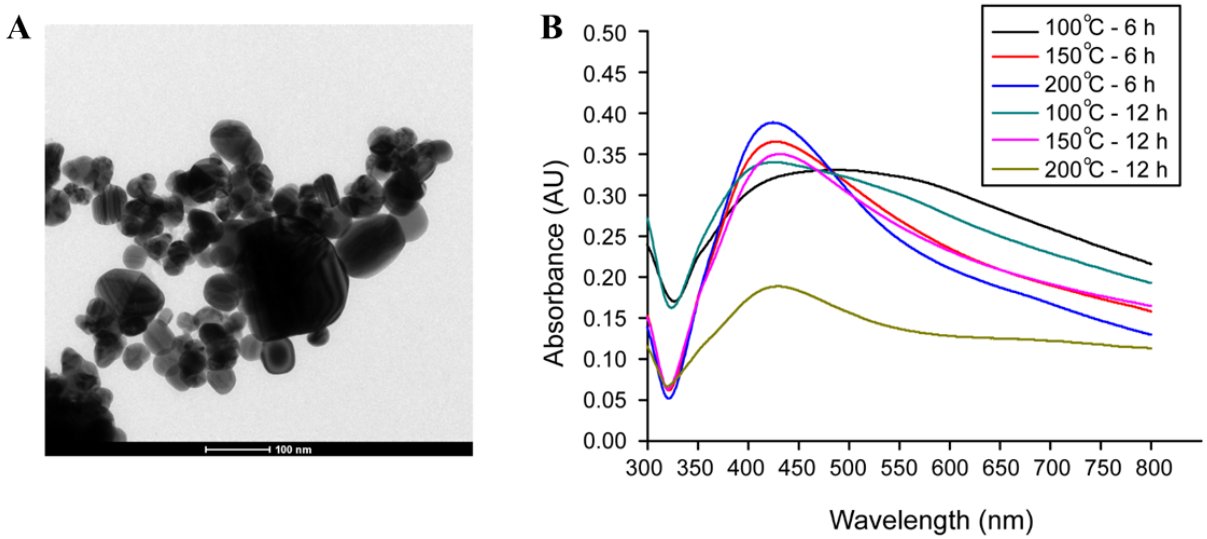

Figure 2 TEM image and UV-vis absorption spectra of AgNPs synthesized using an aloe vera plant-extract solution. TEM image of AgNPs was obtained at 100 ºC for 6 h (A) and UV-vis absorption spectra of AgNPs were shown in the maximum absorption at 420 nm (B).

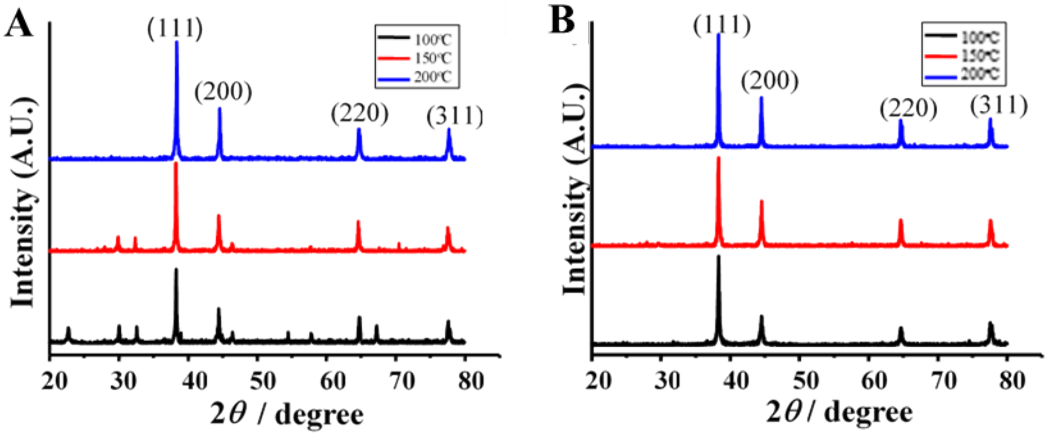

Figure 3 XRD patterns of AgNPs synthesized using an aloe vera plant-extract solution. The AgNPs were prepared at temperatures of 100, 150, and 200 °C and for different times (A) 6 h and (B) 12 h.

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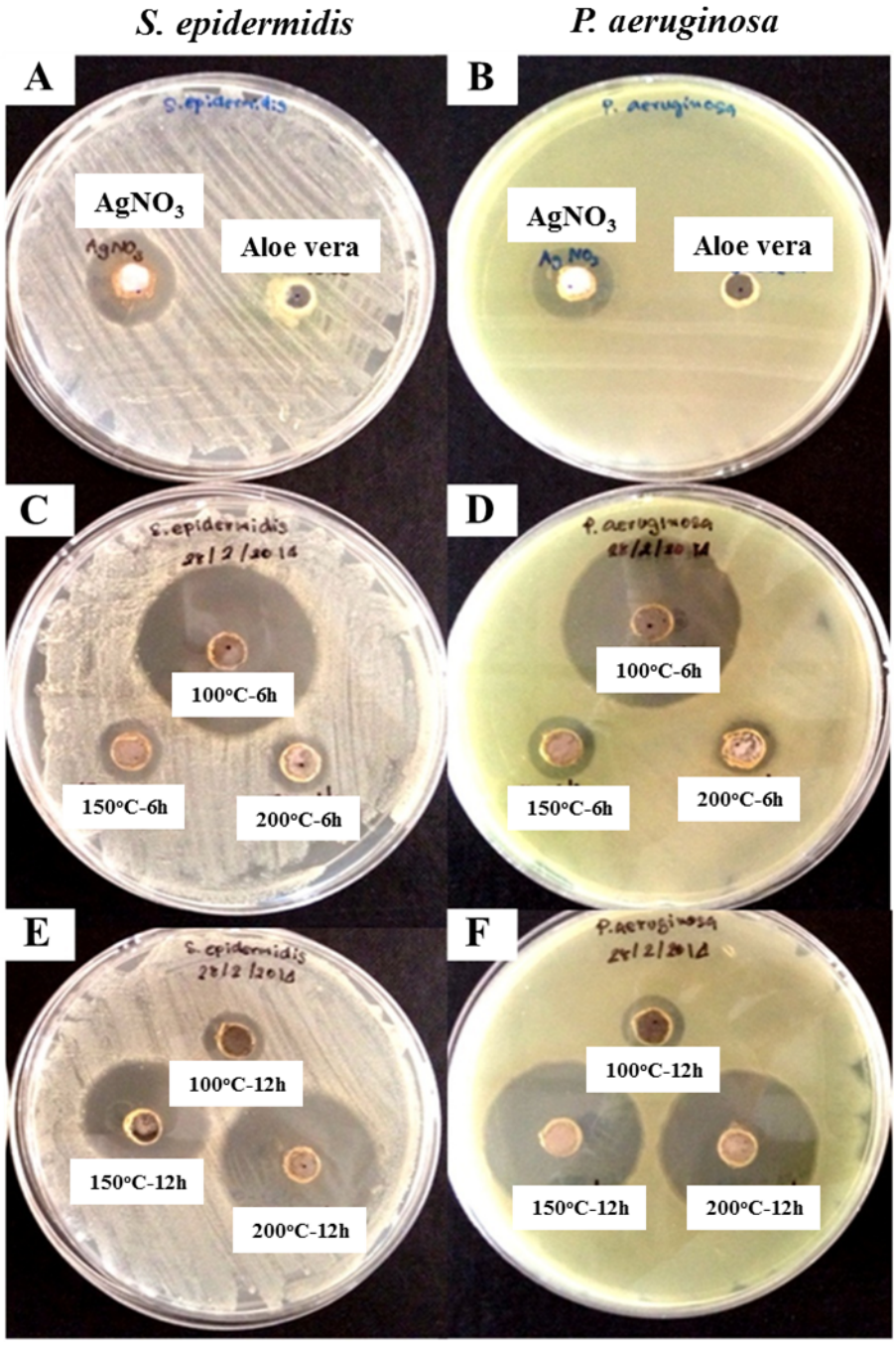

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465 **Figure 4 Antibacterial activity assay of AgNPs against *S. epidermidis* and *P. aeruginosa*.** (A)

466 $AgNO_3$ and aloe-vera extract control in *S. epidermidis*, (B) $AgNO_3$ and aloe-vera extract control

467 in *P. aeruginosa*, (C) 100 °C-6 h, 150 °C-6 h, and 200 °C-6 h AgNPs at (0.1 mg/mL) in *S.*

epidermidis, (D) 100 °C-6 h, 150 °C-6 h, and 200 °C-6 h AgNPs at (0.1 mg/mL) in *P. aeruginosa*, (E) 100 °C-12 h, 150 °C-12 h, and 200 °C-12 h AgNPs at (0.1 mg/mL) in *S. epidermidis*, (F) 100 °C-12 h, 150 °C-12 h, and 200 °C-12 h AgNPs at (0.1 mg/mL) in *P. aeruginosa*.

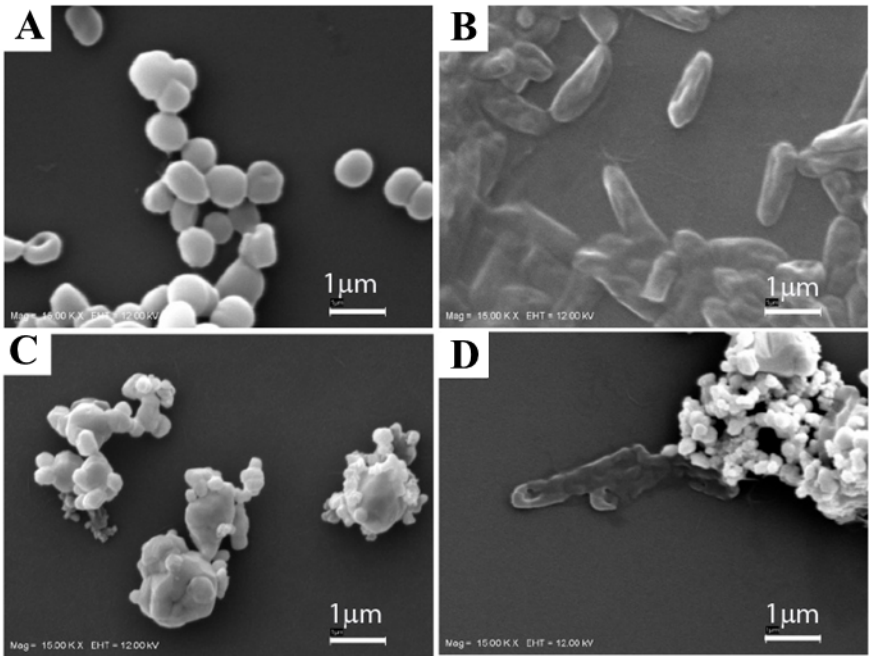

Figure 5 SEM images of the bacterial strains. (A) *S. epidermidis*, (B) *P. aeruginosa*, (C) *S. epidermidis* treated with 100-6 h AgNPs (0.04 mg/mL), (D) *P. aeruginosa* treated with 100-6 h AgNPs (0.04 mg/mL).

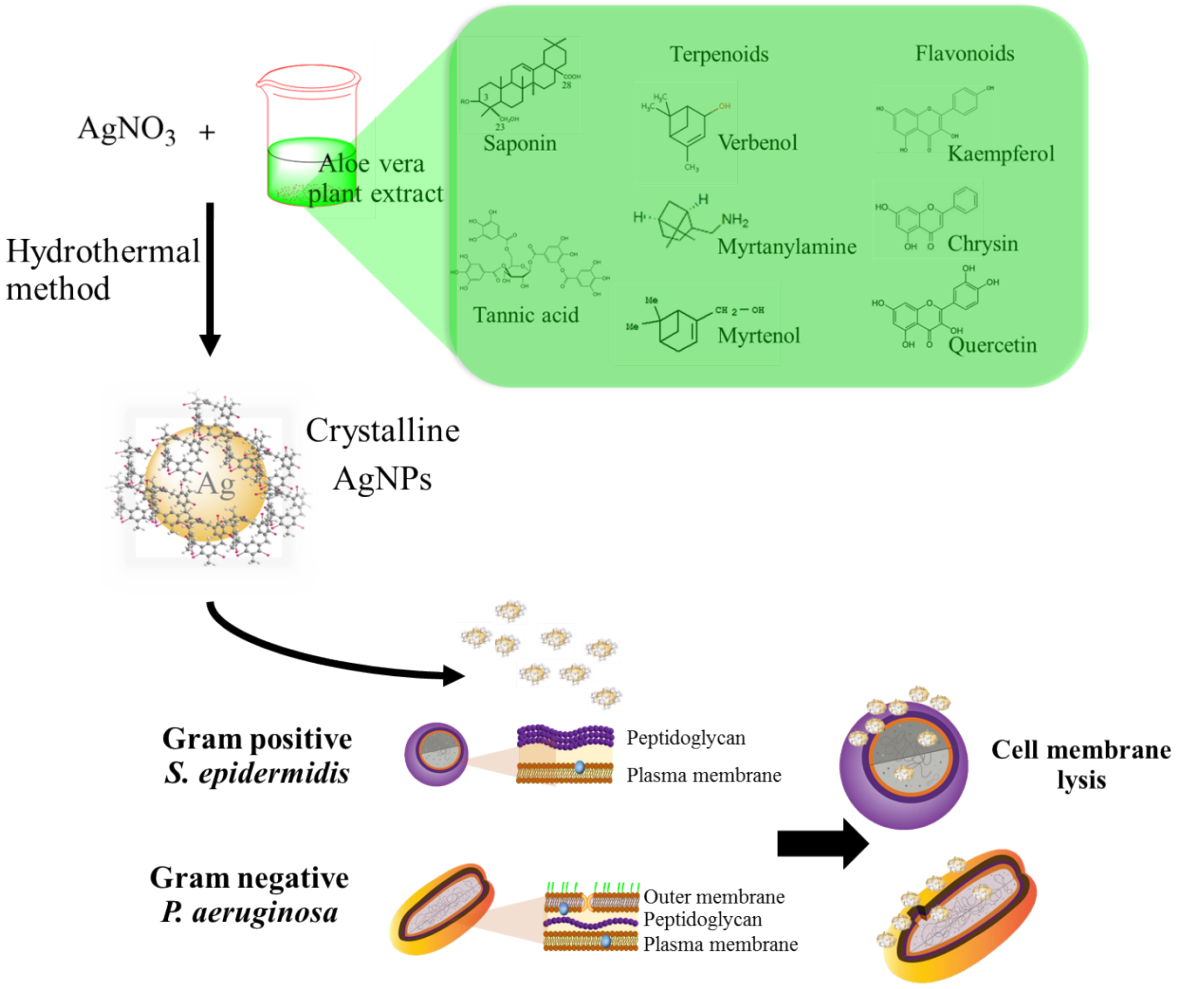

Figure 6 Illustration of proposed bacterial inactivation mechanism that may involve nanocrystalline AgNPs@AV to disrupt the bacterial membrane. In the hydrothermal method, various organic compounds such as saponin, tannin, terpenoids, and flavonoids in the aloe vera

506 plant extract can be combined with $AgNO_3$ synthesizing AgNPs@AV. These nanocrystals may
507 accumulate at the cell membrane increasing its permeability, which eventually results in the death
508 of *P. aeruginosa* and *S. epidermidis*.

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511 **LIST OF TABLE CAPTIONS**

512 **Table 1 Sizes of AgNPs and antibacterial efficiency of AgNPs in different hydrothermal**

513 **processes**.

AgNPs samples	Size of AgNPs (nm)	Inhibition zone diameter (cm)	
		S. epidermidis (gram positive bacteria)	*P. aeroginosa* (gram negative bacteria)
100 °C - 6 h	70.70±22	3.65 ± 0.50*	3.90 ± 0.42*
150 °C - 6 h	79.47±22	1.70 ± 0.43	1.60 ± 0.28
200 °C - 6 h	161.66±53	1.50 ± 0.42	1.40 ± 0.32
100 °C - 12 h	95.25±23	1.72 ± 0.42	1.44 ± 0.29
150 °C - 12 h	149.55±47	3.60 ± 0.56*	3.15 ± 0.49*
200 °C - 12 h	192.02±53	3.90 ± 0.84*	3.45 ± 0.21*

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515 *$p < 0.01$compared with an $AgNO_3$ control

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