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

Patcharaporn Tippayawat, Nutthakritta Phromviyo, Parichart Boueroy, Apiwat Chompoosor

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.

1   **Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a**

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27

28 **Abstract**

29 **Background:** There is worldwide interest in silver nanoparticles (AgNPs) synthesized by

30 various chemical reactions for use in applications exploiting their antibacterial activity, even

31 though these processes exhibit a broad range of toxicity in vertebrates and invertebrates alike.

32 To avoid the chemical toxicity, biosynthesis (green synthesis) of metal nanoparticles is

33 proposed as a cost-effective and environmental friendly alternative. Aloe vera leaf extract is a

34 medicinal agent with multiple properties including an antibacterial effect. Moreover the

35 constituents of aloe vera leaves include lignin, hemicellulose, and pectins which can be used

36 in the reduction of silver ions to produce as AgNPs@aloe vera (AgNPs@AV) with antibacterial

37 activity.

38 **Methods:** AgNPs were prepared by an eco-friendly hydrothermal method using an aloe vera

39 plant extract solution as both a reducing and stabilizing agent. AgNPs@AV were characterized

40 using XRD and SEM. Additionally, an agar well diffusion method was used to screen for

41 antimicrobial activity. MIC and MBC were used to correlate the concentration of AgNPs@AV

42 its bactericidal effect. SEM was used to investigate bacterial inactivation. Then the toxicity

43 with human cells was investigated using an MTT assay.

44 **Results:** The synthesized AgNPs were crystalline with sizes of 70.70 ± 22-192.02 ± 53 nm as

45 revealed using XRD and SEM. The sizes of AgNPs can be varied through alteration of times

46 and temperatures used in their synthesis. These AgNPs were investigated for potential use as

47 an antibacterial agent to inhibit pathogenic bacteria. Their antibacterial activity was tested on

48 *S. epidermidis* and *P. aeruginosa*. The results showed that AgNPs had a high antibacterial

49 which depended on their synthesis conditions, particularly when processed at 100 $^{\circ}$C for 6 h

50 and 200 $^{\circ}$C for 12 h. The cytotoxicity of AgNPs was determined using human PBMCs revealing

51 no obvious cytotoxicity. These results indicated that AgNPs@AV can be effectively utilized

52 in pharmaceutical, biotechnological and biomedical applications.

53   **Discussion:** Aloe vera extract was processed using a green and facile method. This was a

54   hydrothermal method to reduce silver nitrate to AgNPs@AV. Varying the hydrothermal

55   temperature provided the fine spherical shaped nanoparticles. The size of the nanomaterial was

56   affected by its thermal preparation. The particle size of AgNPs could be tuned by varying both

57   time and temperature. A process using a pure AG phase could go to completion in 6 h at 200

58   ᵒC, whereas reactions at lower temperatures required longer times. Moreover, the antibacterial

59   effect of this hybrid nanomaterial was sufficient that it could be used to inhibit pathogenic

60   bacteria since silver release was dependent upon its particle size. The high activity of the largest

61   AgNPs might have resulted from a high concentration of aloe vera compounds incorporated

62   into the AgNPs during hydrothermal synthesis.

63

64   **Introduction**

65   Silver nanoparticles (AgNPs) have been extensively studied for many decades due to their

66   unique features and wide range of applications. Their uses include catalysis *(Pradhan, Pal &*

67   *Pal, 2002)*, biosensing *(Anker et al., 2008)*, imaging *(Lee & El-Sayed, 2006)*, and antibacterial

68   activity *(Morones et al., 2005; Rai, Yadav & Gade, 2009)*. Among these applications,

69   antibacterial activities have gained much attention because they potentially offer a solution to

70   the problem of antibiotic resistance *(Cho et al., 2005)*. There are a variety of methods to

71   synthesize AgNPs including physical and chemical methods *(Chudasama et al., 2010)*.

72   Chemical reduction of silver ions using sodium borohydride *(Zhang et al., 2000)*, hydrazine

73   *(Taleb, Petit & Pileni, 1997)*, ascorbic acid *(Lee et al., 2004)*, trisodium citrate *(Sun Mayers &*

74   *Xia, 2003)*, and polyols *(Sun & Xia, 2002)* were reported and are considered well-established

75   methods. Although chemical routes are effective, these methods may suffer from toxicity due

76   to the chemicals used and the difficulty in removing them. Additionally, chemical reagents

77   used in these methods are hazardous to the environment *(Nabikhan et al., 2010)*. To avoid the

78   toxicity of chemicals, green synthesis was developed *(Sharma, Yngard & Lin, 2009)*. This

79   method of biosynthesis of metal nanoparticles has been proposed as a cost-effective and

80   environmental friendly way of fabricating these materials.

81   Synthesis of AgNPs employing either microorganisms or plant extracts has emerged as an

82   alternative approach. These biosynthetic methods have a numbers of benefits. They are simple,

83   cost-effective, give high yields, and are environmentally friendly *(Zhang et al., 2013)*. Plant

84   extracts have reportedly been used in the preparation of AgNPs *(Sun et al., 2014)*. Aloe vera

85   leaves have been used as medicinal plants since they possess anti-inflammatory activity, UV

86   protection, anti-arthritic properties, promote wound and burn-healing, and have antibacterial

87   properties *(Chandran et al., 2006; Feng et al., 2000; Reynolds & Dweck, 1999; Vazquez et al.,*

88   *1996)*. There are a number of biologically active constituents in aloe vera leaves. These include

89   lignin, hemicellulose, pectins which can be used in the reduction of silver ions *(Emaga et al.,*

90   *2008)*. It is believed that the large enzymes and proteins in aloe vera extract are weakly bound

91   to silver ions and function as a complexing agent. Due to their low cost and environmentally

92   friendly nature coupled with their reducing properties, we selected aloe vera as the reducing

93   and stabilizing agent to prepare AgNPs and test their antibacterial activity.

94   In this study, we report a one-step hydrothermal method to prepare silver nanoparticles.

95   Reduction of Ag^+ ions to Ag^0 nanoparticles was done in a medium of aloe vera extract in which

96   no extra reducing agent was used. This method is considered green synthesis. The resulting

97   AgNPs can be obtained in large quantities. The sizes of AgNPs were found to be in a range of

98   70.70-192.02 nm and controllable by varying temperature and time conditions of the

99   hydrothermal process. Further, the resulting AgNPs were found to be effective against gram-

100   positive (*Streptococcus epidermidis*) and gram-negative (*Pseudomonas aeruginosa*).

101

102   **Materials and Methods**

103   In this study, silver nitrate, AgNO₃ (Sigma-Aldrich Chemicals, USA) and aloe vera plant

104   extract were used as the starting materials. The aloe vera extract solution was prepared using

105   50 g of aloe vera leaves that had been rinsed with deionized water and finely cut into small

106   pieces. The chopped aloe vera leaves were boiled in a 50 mL of deionized water for 20 minutes

107   and allowed to cool. The cooled leaf broth was filtered and stored in a refrigerator at 4 °C. The

108   resulting extract was used as an aloe vera extract solution.

Synthesis of AgNPs and Characterization of AgNPs

110   In the preparation of AgNPs samples, AgNO₃ (0.3 mol) was first dissolved in 20 ml of

111   deionized water and mixed with 20 ml of aloe vera extract solution under vigorous stirring at

112   room temperature for 30 minutes. The mixtures were added to sealed Teflon-lined vessels of

113   100 mL capacity (Parr, USA), which were heated and maintained at various time and

114   temperature conditions, and then gradually cooled to room temperature. A gray precipitate was

115   collected by filtration and washed with deionized water several times, and finally dried in air

116   at 60 °C for 6 h. The crystal phase analysis of the AgNPs powders was conducted using X-ray

117   diffraction (XRD) (PW3710, the Netherlands) with CuKα radiation (λ = 0.15406 nm). The

118   particle sizes and morphology of the prepared AgNPs samples were characterized using

119   scanning electron microscopy (SEM) (LEO SEM 1450VP, UK).

Antibacterial Tests and Cytotoxicity Test

Well diffusion method

122   The antibacterial activity of AgNPs prepared under different hydrothermal processing

123   conditions were tested against gram-negative *P. aeruginosa* (*Pseudomonas aeruginosa*,

124   ATCC27803) and gram-positive *S. epidermidis* (*Staphylococcus epidermidis*, ATCC35984)

125   using an agar well diffusion method. The organisms were sub-cultured in nutrient broth at 37

126   °C and incubated overnight. After that, Nutrient Agar (Merck) was swabbed with the respective

127   sub-cultures (1×10^8 CFU/ml). Specimens containing AgNPs were then arranged on the

128 swabbed agar surface and incubated at 37 °C for 24 h. The results were read by measuring the

129 diameter of the inhibition zone (mm). The experiments were done in triplicate.

Scanning electron microscopy (SEM)

131 Scanning electron microscopy of control cells and AgNPs treated cells (0.04 mg/mL) was

132 performed to investigate the antibacterial activity. Each bacterial culture was prepared as

133 described above and then pipetted into a 6-well plate with and without AgNPs prior to covering

134 the wells with glass slides. After incubating at 37 °C overnight, the glass slides were removed

135 and gently washed with phosphate buffer saline 3 times before dehydration in an alcohol series

136 using concentrations of 25%, 50%, 75%, 90% and 100% ethanol in distilled water. The slides

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

138 until analysis.

Minimum inhibitory concentration (MIC) and minimal bactericidal concentration

(MBC)

141 A microdilution method was used to indicate the bactericidal effect of AgNPs. A suspension

142 of 1×10^8 CFU/ml of bacteria in nutrient broth was prepared as described above. The

143 antibacterial solutions were prepared using serial two-fold (1:2) dilutions of AgNPs in

144 concentrations ranging from 0.04 to 0.00008 mg/mL and incubated at 37 °C for 24 h. In the

145 range of sample turbidity, the MIC of the samples could not be determined to identify the

146 lowest concentration of antibacterial agent that inhibits 99% of the growth of the bacteria. A

147 microdilution measurement was done in triplicate to confirm the value of MIC for each tested

148 bacteria. As such, the MBC was measured after MIC determination. In this assay, 10 μl from

149 all concentrations of AgNPs were pipetted onto nutrient agar plates and incubated at 37 °C for

150 24 h. The MBC endpoint was interpreted at the lowest concentration of antibacterial agent

151 killing 100% of the initial bacterial population.

Cytotoxicity Test

153   The AgNP samples produced at100 ºC for 6 h and 200 ºC for 12 h were tested for their

154   cytotoxicity using the MTT3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

155   assay. Human peripheral blood mononuclear cells (PBMCs) from the leftover buffy coat were

156   suspended into complete 1640 RPMI (supplemented with 10% fetal bovine serum, 2 mM L-

157   glutamine, 100 unit/ml penicillin and 100 µg/ml streptomycin) in a 96-well plate at a density

158   of 10^5 cells/well. This was done prior to exposure to AgNPs dissolved in RPMI to make a stock

159   concentration at 0.04 mg/mL. The stock solution was used to generate serial two-fold dilution

160   at 4 concentrations, i.e., 0.02, 0.01, 0.005, and 0.0025 mg/mL. Then, the cells were incubated

161   at 37 ºC in a fully humidified, 5% CO_2 air atmosphere for 48 h. The test samples were removed

162   from the cell cultures and the cells were reincubated for a further 24 h in fresh medium. They

163   were then tested using the MTT assay. Briefly, 50 µl of MTT in phosphate buffered saline at 5

164   mg/ml was added into a medium in each well and the cells were incubated for 4 h. The medium

165   and MTT were then gently aspirated from the wells and solubilized in formazan with 200 µl of

166   DMSO and 25 µl of Sorensen's Glycine buffer, pH 10.5. The optical density was read with a

167   microplate reader at a wavelength of 560 nm. The average of 3 wells was used to determine

168   the mean of each point. Then % survival of the cells was calculated. For each test sample, the

169   data was used to determine the concentration of sample required to kill 50% (IC_{50}) of the cells

170   compared to that of the controls. A dose-response curve was derived from 5 concentrations in

171   the test range using 3 wells per concentration.

172

173   **Results**

174   **Characterization of Silver Nanoparticles**

175   The morphology of AgNPs prepared at different reaction temperatures and times was examined

176   using SEM. The result showed SEM images of AgNPs obtained by the reduction of $AgNO_3$

177   with aloe vera plant extract (Fig. 1). It was found that the reaction time and temperature had

178   significant effects on the formation of Ag nanostructures. AgNPs were observed as spherical

179   particles with the sizes between 70.7-192.02 nm, moreover the sizes of the materials were

180   significantly affected by their preparation temperature as presented in Table 1. At 6 h, the

181   AgNPs showed the sizes of 70.70 ± 23, 79.47 ± 22, and 161.66 ± 53 nm prepared at 100 ºC,

182   150 ºC and 200 ºC, respectively. At 12 h, the AgNPs showed sizes of 95.25 ± 23, 149.55 ± 47

183   and 192.02 ± 53 nm prepared at 100 ºC, 150 ºC and 200 ºC, respectively. The XRD patterns of

184   AgNPs resulted from using the above 3 hydrothermal conditions (Figs. 2A and 2B). All of the

185   main peaks were indexed as AgNPs with the face centered cubic (fcc) lattice of silver, as shown

186   in the standard data (JCPDS file No.01-071-4613). The diffraction peaks at 2θ degree of 38.2,

187   44.3, 64.5 and 77.1 corresponded to the (111), (200), (220), and (311) planes, respectively. A

188   pure phase of Ag was only obtained at a temperature of 200 ºC for 6 h. The chemical reaction

189   to form a pure phase at 100 and 150 ºC for 6 h was incomplete because reaction at such a low

190   temperature usually requires a longer time (Fig. 2A). The existence of Ag_2O was shown at the

191   peak at around 31.9 *(Liu et al., 2010)*. The result showed a pure Ag phase in all the samples

192   prepared using hydrothermal conditions for 12 h (Fig. 2b).

193   **Antibacterial Effects**

194   An advantage of silver nanoparticles is that they are known to have an antibacterial effect *(Rai*

195   *et al., 2012)*. However, the AgNPs formed during the aloe-vera hydrothermal method,

196   AgNPs@AV, need to have bioactive functions. It is especially important to understand the

197   functional effects on microorganisms in order to develop novel antibacterial agents. To

198   demonstrate this activity, AgNPs were studied for their bactericidal effect against pathogenic

199   gram-positive *S. epidermidis* and gram-negative *P. aeruginosa*. This was done using a

200   qualitative antibacterial well diffusion assay and studying AgNPs interaction with bacteria

201   using SEM. Quantitative antibacterial concentrations were evaluated by determining the

202   minimum bactericidal concentration (MBC). It was observed that the inhibition zones of both

203   pathogens were significant for 0.1 mg/mL AgNPs prepared at 100 ℃ for 6 h, 150 ℃ and 200 ℃

204   for 12 h compared with the control (Fig. 3 and Table 1). The AgNPs synthesized under different

205   conditions provided varying bactericidal effects. Then, the effects of two AgNPs@AV samples

206   including those prepared at 100 ℃ for 6 h and 200 ℃ for 12 h, were selected for further studies

207   using SEM and MBC. The interaction of AgNPs and microorganisms was shown using SEM.

208   The result indicated the cell membrane changed when contacted with the nanoparticles (Fig.

209   4). This was particularly true for gram-negative bacteria, showing a thin layer of membrane

210   and having pores. Subsequently, MBC was determined for both *S. epidermidis* and *P.*

211   *aeruginosa*. This demonstrated the lowest concentration of nanoparticles with bactericidal

212   effect was 0.01 mg/mL for AgNPs fabricated at 100 ℃ for 6 h and 200 ℃ for 12 h against *S.*

213   *epidermidis*. The corresponding concentrations was 0.0025 mg/mL for AgNPs fabricated at

214   100 ℃ for 6 h and 0.00125 mg/mL for those formed at 200 ℃ for 12 h against *P. aeruginosa*.

215   At the lower AgNPs concentrations, clearly there was an effect on the lethality against gram

216   negative-bacteria whereas higher concentrations were needed to control gram-positive bacteria.

217   **Cytotoxicity Evaluation**

218   To determine the cytotoxicity of AgNPs@AV on human cells, PBMCs were tested using the

219   MTT assay. The result was calculated as %survival of the cells cultured with samples at

220   concentrations of 0.04, 0.02, 0.01, 0.005, and 0.0025 mg/mL of 100 ℃ for 6 h and 200 ℃ for

221   12 h processed AgNPs@AV. The %survival of the cells in less 0.0025 mg/mL of both

222   nanoparticles was significantly higher than 50% which confirms that these AgNPs@AVs were

223   non-toxic to human PBMCs. Nanoparticles produced by green synthesis can be useful in

224   biomedical applications.

225

226   **Discussion**

227 Recently, there has been increasing study of AgNPs synthesis to develop several applications

228 such as catalysis, biosensing, imaging, and antibacterial activity. Green synthesis is an

229 alternative method developed to produce metal nanoparticles by using natural compounds or

230 plant components. These are environmentally friendly processes that avoid the toxicity of

231 chemicals. Algae, bacteria, fungi and plants have been used to synthesize NPs without the need

232 for additional reducing and stabilizing agents. Plant extracts contain functional substances,

233 including cyclic peptides, sorbic acid, citric acid, euphol, polyhydroxy limonoids, ascorbic

234 acid, retinoic acid, tannins, ellagic acid, and gallic acid, among others, are strongly believed to

235 play a crucial role in the bioreduction and stabilization of nanoparticles *(Rajan et al., 2015)*.

236 These processes seem facile, safe, low cost, and ecofriendly, eliminating the elaborate process

237 of maintaining aseptic cell cultures and are suitable for large scale production. Therefore, this

238 study focused on the biosynthesis of AgNPs with plant extracts of aloe vera leaves. Zhang et

239 al. (*2010*) speculated that the hydroquinones in the aloe vera plant extract act as the reducing

240 agents. Additionally, the spherical shape of AgNPs was governed by the weaker binding of

241 proteins in the solution leading to the isotropic growth of the AgNPs. Here, the hydrothermal

242 process was applied to AgNPs synthesis in which time and temperature had an effect on the

243 resulting crystalline structure of AgNPs. High temperature and pressure are necessary to

244 facilitate the reduction processes *(Liu et al., 2012)*. Nucleation and the growth of AgNPs

245 depend on the reaction temperature. Additionally, capping agents also play a role in the

246 synthesis of nanoparticles. Selective interaction of capping agents may lead to anisotropic

247 crystalline growth. Poly (vinyl) pyrrolidoneis are widely used to synthesize nanorods due to

248 their preferential interaction with the (100) plane *(Pal, Tak & Song, 2007)*. In the case of aloe

249 vera, a (111) plane of AgNPs predominantly arose as a major peak. This plane was reported

250 responsible for a strong antibacterial effect *(Feng et al., 2000)*.

251   The factors controlling the morphology, size, and product purity in the hydrothermal process

252   were reaction temperature and time *(Byrappa & Adschiri, 2007; Liu et al., 2014)*. Moreover,

253   biosynthesis of inorganic nanoparticles with the plant extracts improved their bactericidal

254   effect *(Yousefzadi, Rahimi & Ghafori, 2014)*. High bactericidal activity was possibly caused

255   by synergistic antibacterial effects of AgNPs and naturally-occurring chemicals in aloe vera.

256   The lethal mechanism against pathogenic *S. epidermidis* and *P. aeruginosa* might involve the

257   release of Ag^+ ions from AgNPs and the formation of crystalline bio-organic compounds of

258   aloe vera plant extract assembled with AgNPs anchored onto the bacterial cell walls, producing

259   pits and penetrating into the cytoplasm. Various natural ligands can interact with microbial

260   membrane such as saponin, tannin, terpenoids, and flavonoids in the aloe vera *(Griffin et al.,*

261   *1999; Sahu et al., 2013)*. The interaction with the cell membrane may increase its permeability

262   leading to cell lysis. Moreover the free radicals from metal result in induction of oxidative

263   stresses, such as reactive oxygen species (ROS), that can damage the bacterial membranes,

264   mitochondria, and DNA. This eventually results in the death of the cell *(Hajipour et al., 2012;*

265   *Tamboli & Lee, 2014)*. From our results, a schematic mechanism involving the reaction of

266   AgNPs@AV to kill the bacteria was purposed and illustrated in Figure 5. Additionally, the

267   susceptibility of different types of bacteria was attributed to the structure of their bacterial cell

268   walls. Previous studies indicated that the silver ion released from AgNPs was responsible for

269   antibacterial activity *(Feng et al., 2000)*. The free silver ion can then bind with the thiol groups

270   of enzymes *(Zhang et al., 2013)*. The AgNPs formed at 100 ºC for 6 h were found to be toxic

271   to both gram-positive and gram-negative bacteria. This might due to the smaller size of the

272   AgNPs fabricated under these conditions which results a higher surface area *(Cui et al., 2013)*.

273   Silver ion release is a size dependent process *(Cui et al., 2013)*. The antibacterial activity of

274   the synthesized AgNPs might be due to the silver ion release and the resulting genotoxic

275   activity of aloe vera on *E. coli (Zhang et al., 2010)*. Interestingly, the samples processed at 200

276 ºC for 12 h had the largest size of those examined and they provided effective growth inhibition

277 of the pathogens. The results indicated that the larger AgNPs might contain high levels of

278 incorporated aloe vera compounds as well as a pure Ag phase due their long time and high

279 temperature treatment. Therefore, this hybrid nanostructure formed under specific conditions

280 can potential be an antibacterial agent.

281

282 **Conclusion**

283 This report described a green and facile method to synthesize AgNPs in large quantities. Silver

284 nitrate was reduced in an aloe vera plant-extract solution under a hydrothermal condition. Aloe

285 vera plant extract solutions were used as both reducing and stabilizing agents. Fine spherically

286 shape nanoparticles were obtained. The particle size of AgNPs can be tuned by varying the

287 hydrothermal temperature. The antibacterial effect of AgNPs@AV showed promise for use as

288 a highly potent agent with minimal cytotoxicity to human PBMCs. These hybrid nanomaterials

289 could potentially be used in biomedical applications.

290

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299

300 **References:**

301   **Anker JN, Hall WP, Lyandres O, Shah NC, Zhao J, Van Duyne RP. 2008.** Biosensing with

302   plasmonic nanosensors. *Nature materials* **7**,442-453.

303   **Byrappa K, Adschiri T. 2007.** Hydrothermal technology for nanotechnology. *Progress in*

304   *Crystal Growth and Characterization of Materials* **53**,117-166.

305   **Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M. 2006.** Synthesis of gold

306   nanotriangles and silver nanoparticles using Aloevera plant extract. *Biotechnology*

307   *progress* **22**,577-583.

308   **Cho K-H, Park J-E, Osaka T, Park S-G. 2005.** The study of antimicrobial activity and

309   preservative effects of nanosilver ingredient. *Electrochimica Acta* **51**,956-960.

310   **Chudasama B, Vala AK, Andhariya N, Mehta R, Upadhyay R. 2010.** Highly bacterial

311   resistant silver nanoparticles: synthesis and antibacterial activities. *Journal of*

312   *Nanoparticle Research* **12**,1677-1685.

313   **Cui L, Chen P, Chen S, Yuan Z, Yu C, Ren B, Zhang K. 2013.** In situ study of the

314   antibacterial activity and mechanism of action of silver nanoparticles by surface-

315   enhanced Raman spectroscopy. *Analytical chemistry* **85**,5436-5443.

316   **Emaga TH, Robert C, Ronkart SN, Wathelet B, Paquot M. 2008.** Dietary fibre components

317   and pectin chemical features of peels during ripening in banana and plantain varieties.

318   *Bioresource Technology* **99**,4346-4354.

319   **Feng Q, Wu J, Chen G, Cui F, Kim T, Kim J. 2000.** A mechanistic study of the antibacterial

320   effect of silver ions on Escherichia coli and Staphylococcus aureus. *Journal of*

321   *biomedical materials research* **52**,662-668.

322   **Griffin SG, Wyllie SG, Markham JL, Leach DN. 1999.** The role of structure and molecular

323   properties of terpenoids in determining their antimicrobial activity. *Flavour and*

324   *Fragrance Journal* **14**,322-332.

325   **Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo**

326   **T, Serpooshan V, Parak WJ, Mahmoudi M. 2012.** Antibacterial properties of

327   nanoparticles. *Trends in biotechnology* **30**,499-511.

328   **Lee G-J, Shin S-I, Kim Y-C, Oh S-G. 2004.** Preparation of silver nanorods through the

329   control of temperature and pH of reaction medium. *Materials Chemistry and Physics*

330   **84**,197-204.

331   **Lee K-S, El-Sayed MA. 2006.** Gold and silver nanoparticles in sensing and imaging:

332   sensitivity of plasmon response to size, shape, and metal composition. *The Journal of*

333   *Physical Chemistry B* **110**,19220-19225.

334   **Liu J, Sonshine DA, Shervani S, Hurt RH. 2010.** Controlled release of biologically active

335   silver from nanosilver surfaces. *ACS nano* **4**,6903-6913.

336   **Liu Z, Wang Y, Zu Y, Fu Y, Li N, Guo N, Liu R, Zhang Y. 2014.** Synthesis of

337   polyethylenimine (PEI) functionalized silver nanoparticles by a hydrothermal method

338   and their antibacterial activity study. *Materials Science and Engineering: C* **42**,31-37.

339   **Liu Z, Xing Z, Zu Y, Tan S, Zhao L, Zhou Z, Sun T. 2012.** Synthesis and characterization

340   of L-histidine capped silver nanoparticles. *Materials Science and Engineering: C*

341   **32**,811-816.

342   **Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ.**

343   **2005.** The bactericidal effect of silver nanoparticles. *Nanotechnology* **16**,2346.

344   **Nabikhan A, Kandasamy K, Raj A, Alikunhi NM. 2010.** Synthesis of antimicrobial silver

345   nanoparticles by callus and leaf extracts from saltmarsh plant, Sesuvium portulacastrum

346   L. *Colloids and Surfaces B: Biointerfaces* **79**,488-493.

347   **Pal S, Tak YK, Song JM. 2007.** Does the antibacterial activity of silver nanoparticles depend

348   on the shape of the nanoparticle? A study of the gram-negative bacterium Escherichia

349   coli. *Applied and environmental microbiology* **73**,1712-1720.

350   **Pradhan N, Pal A, Pal T. 2002.** Silver nanoparticle catalyzed reduction of aromatic nitro

351   compounds. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*

352   **196**,247-257.

353   **Rai M, Deshmukh S, Ingle A, Gade A. 2012.** Silver nanoparticles: the powerful nanoweapon

354   against multidrugî€• resistant bacteria. *Journal of applied microbiology* **112**,841-852.

355   **Rai M, Yadav A, Gade A. 2009.** Silver nanoparticles as a new generation of antimicrobials.

356   *Biotechnology advances* **27**,76-83.

357   **Rajan R, Chandran K, Harper SL, Yun S-I, Kalaichelvan PT. 2015.** Plant extract

358   synthesized silver nanoparticles: An ongoing source of novel biocompatible materials.

359   *Industrial Crops and Products* **70**,356-373.

360   **Reynolds T, Dweck A. 1999.** Aloe vera leaf gel: a review update. *Journal of*

361   *ethnopharmacology* **68**,3-37.

362   **Sahu PK, Giri DD, Singh R, Pandey P, Gupta S, Shrivastava AK, Kumar A, Pandey KD.**

363   **2013.** Therapeutic and medicinal uses of Aloe vera: a review. *Pharmacology &*

364   *Pharmacy* **4**,599.

365   **Sharma VK, Yngard RA, Lin Y. 2009.** Silver nanoparticles: green synthesis and their

366   antimicrobial activities. *Advances in colloid and interface science* **145**,83-96.

367   **Sun Q, Cai X, Li J, Zheng M, Chen Z, Yu C-P. 2014.** Green synthesis of silver nanoparticles

368   using tea leaf extract and evaluation of their stability and antibacterial activity. *Colloids*

369   *and Surfaces A: Physicochemical and Engineering Aspects* **444**,226-231.

370   **Sun Y, Mayers B, Xia Y. 2003.** Transformation of silver nanospheres into nanobelts and

371   triangular nanoplates through a thermal process. *Nano Letters* **3**,675-679.

372   **Sun Y, Xia Y. 2002.** Shape-controlled synthesis of gold and silver nanoparticles. *Science*

373   **298**,2176-2179.

374   **Taleb A, Petit C, Pileni M. 1997.** Synthesis of highly monodisperse silver nanoparticles from

375   AOT reverse micelles: a way to 2D and 3D self-organization. *Chemistry of Materials*

376   **9**,950-959.

377   **Tamboli DP, Lee DS. 2014.** Mechanistic antimicrobial approach of extracellularly synthesized

378   silver nanoparticles against gram positive and gram negative bacteria. *Journal of*

379   *hazardous materials* **260**,878-884.

380   **Vazquez B, Avila G, Segura D, Escalante B. 1996.** Antiinflammatory activity of extracts

381   from Aloe vera gel. *Journal of ethnopharmacology* **55**,69-75.

382   **Yousefzadi M, Rahimi Z, Ghafori V. 2014.** The green synthesis, characterization and

383   antimicrobial activities of silver nanoparticles synthesized from green alga

384   Enteromorpha flexuosa (Wulfen) J. Agardh. *Materials Letters* **137**,1-4.

385   **Zhang Y, Cheng X, Zhang Y, Xue X, Fu Y. 2013.** Biosynthesis of silver nanoparticles at

386   room temperature using aqueous aloe leaf extract and antibacterial properties. *Colloids*

387   *and Surfaces A: Physicochemical and Engineering Aspects* **423**,63-68.

388   **Zhang Y, Yang D, Kong Y, Wang X, Pandoli O, Gao G. 2010.** Synergetic antibacterial

389   effects of silver nanoparticles@ aloe vera prepared via a green method. *Nano Biomed*

390   *Eng* **2**,252-257.

391   **Zhang Z, Patel RC, Kothari R, Johnson CP, Friberg SE, Aikens PA. 2000.** Stable silver

392   clusters and nanoparticles prepared in polyacrylate and inverse micellar solutions. *The*

393   *Journal of Physical Chemistry B* **104**,1176-1182.

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397 **LIST OF FIGURES AND LEGENDS**

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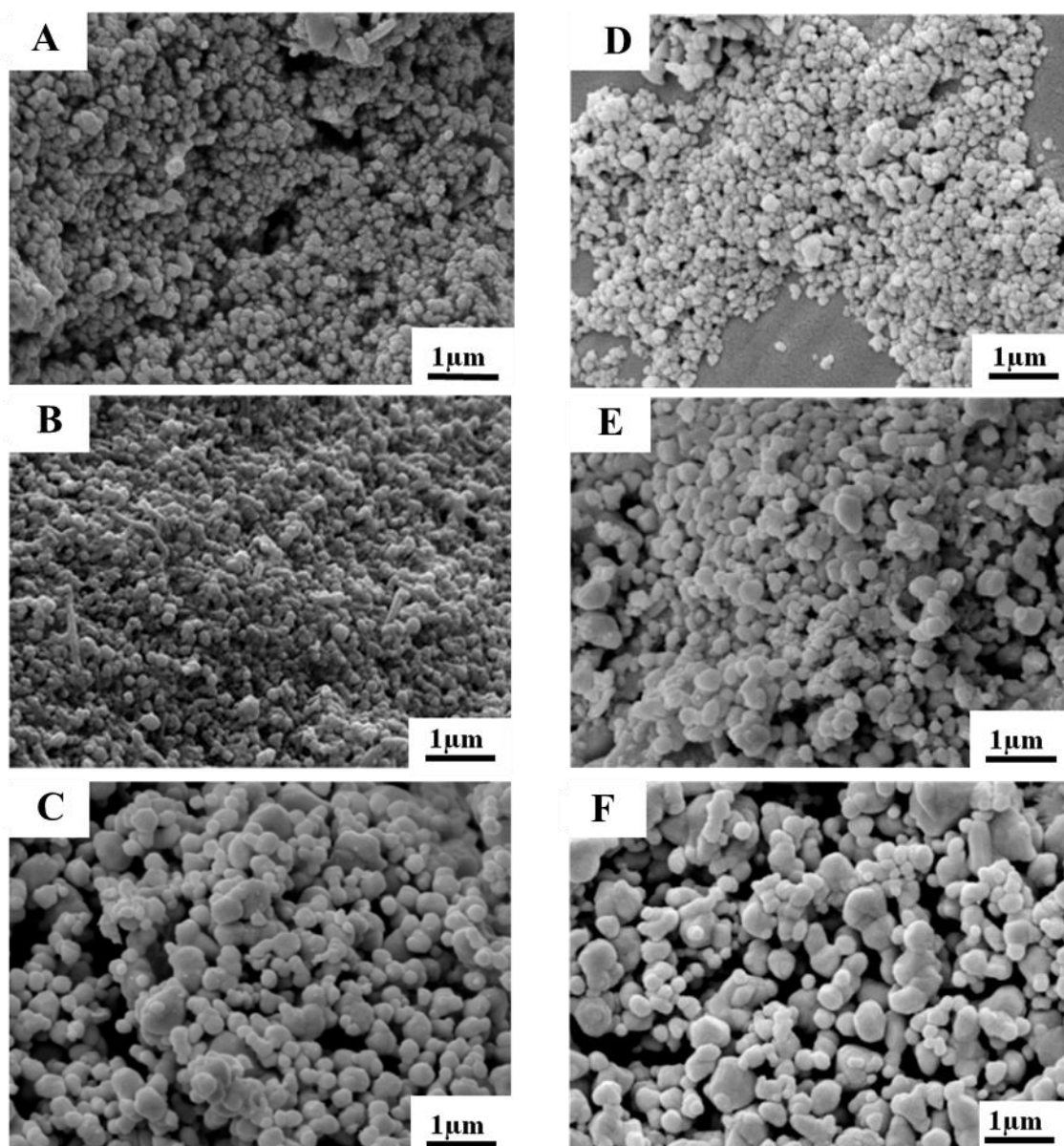
399 **Figure 1 SEM images of silver nanoparticles on a glass slide after incubation at different**

400 **temperature and time combinations.** SEM images of AgNPs were obtained at (A) 100 ºC

401 for 6 h, (B) 150 ºC for 6 h, (C) 200 ºC for 6 h, (D) 100 ºC for 12 h, (E) 150 ºC for 12 h and

402 (F) 200 ºC for 12 h.

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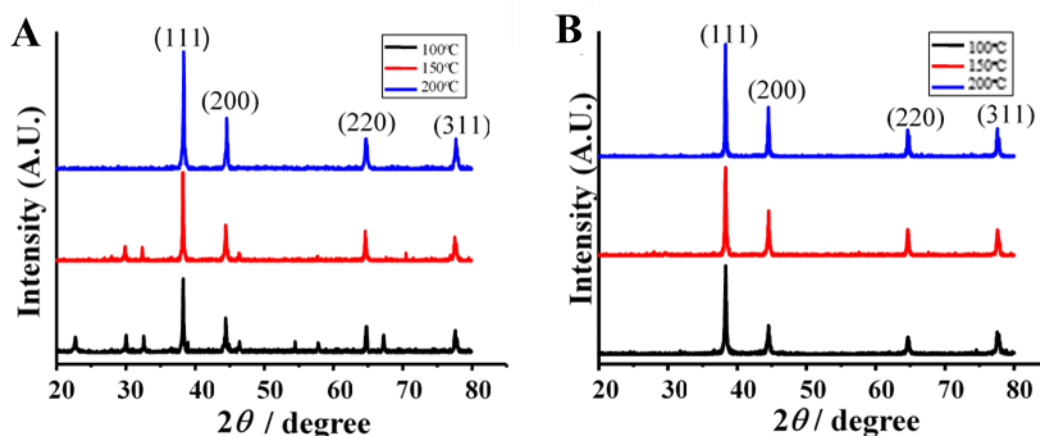
408 **Figure 2 XRD patterns of AgNPs synthesized using an aloe vera plant-extract solution.**

409 The AgNPs were prepared at temperatures of 100, 150, and 200 ºC and for different times (A)

410 6 h and (B) 12 h.

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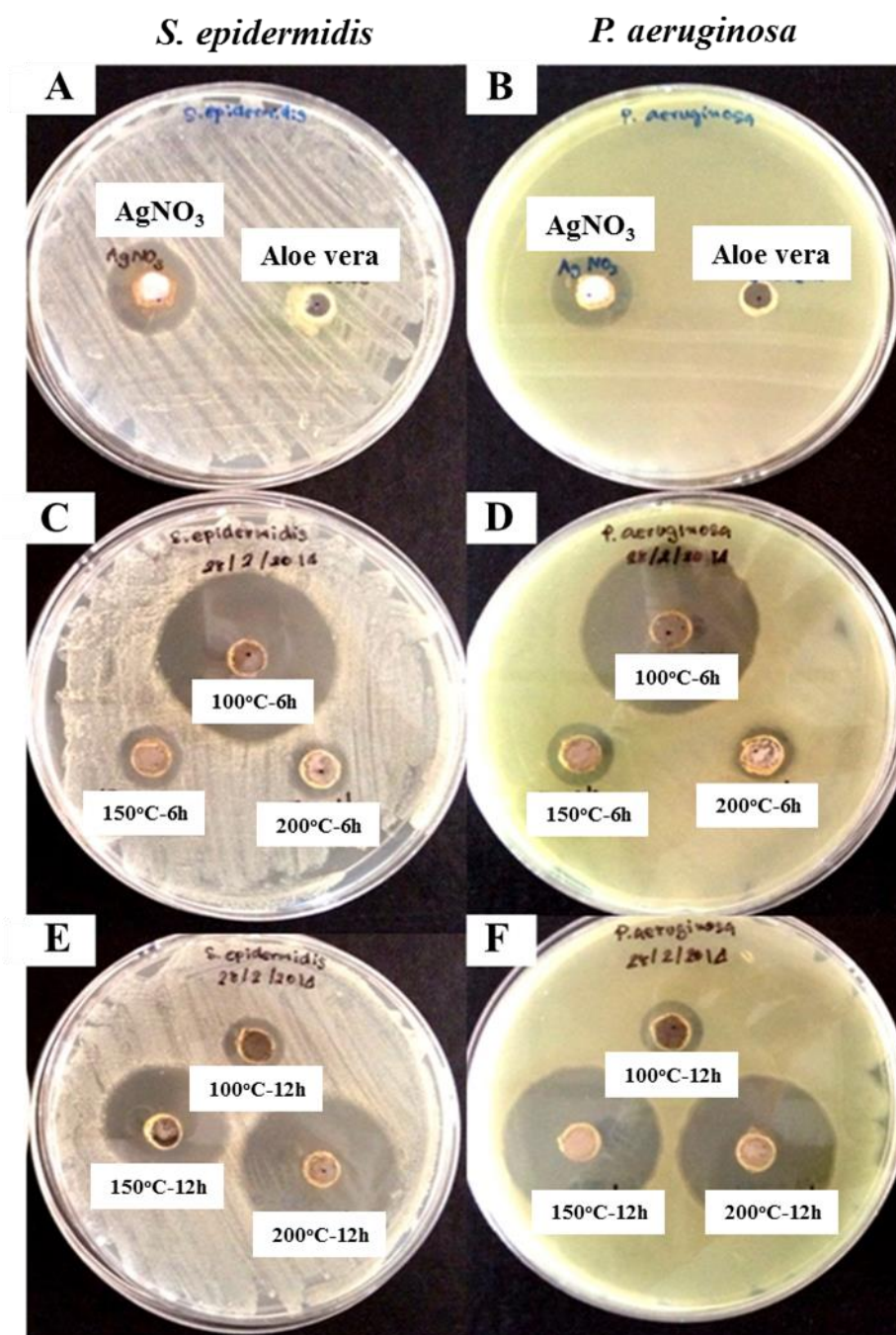


Figure 3 Antibacterial activity assay of AgNPs against *S. epidermidis* and *P. aeruginosa*.

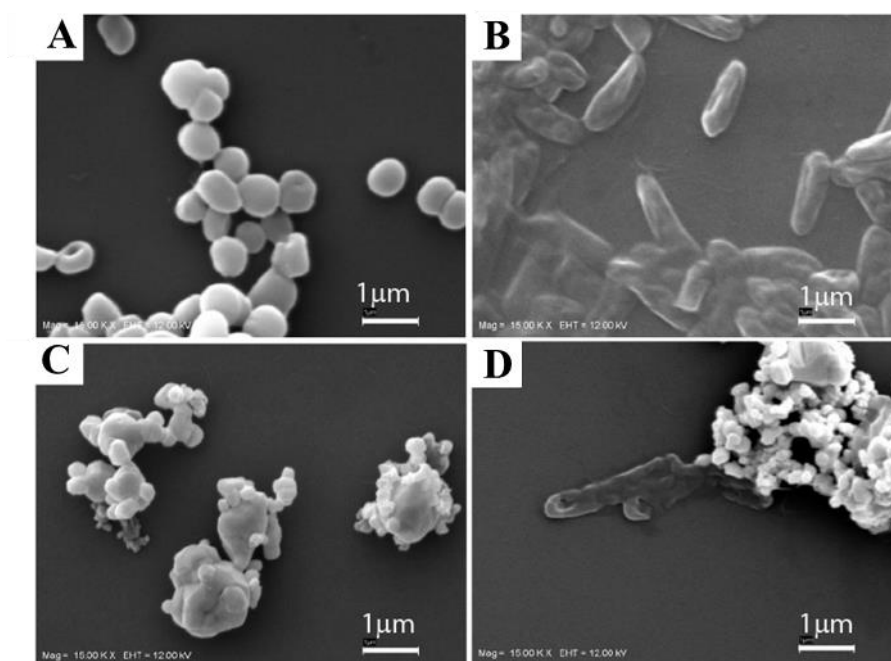
(A) $AgNO_3$ and aloe-vera extract control in *S. epidermidis*, (B) $AgNO_3$ and aloe-vera extract

control 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.

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452 **Figure 4 SEM images of the bacterial strains.** (A) *S. epidermidis*, (B) *P. aeruginosa*,

453 (C) *S. epidermidis* treated with 100-6 h AgNPs (0.04 mg/mL), (D) *P. aeruginosa* treated with

454 100-6 h AgNPs (0.04 mg/mL).

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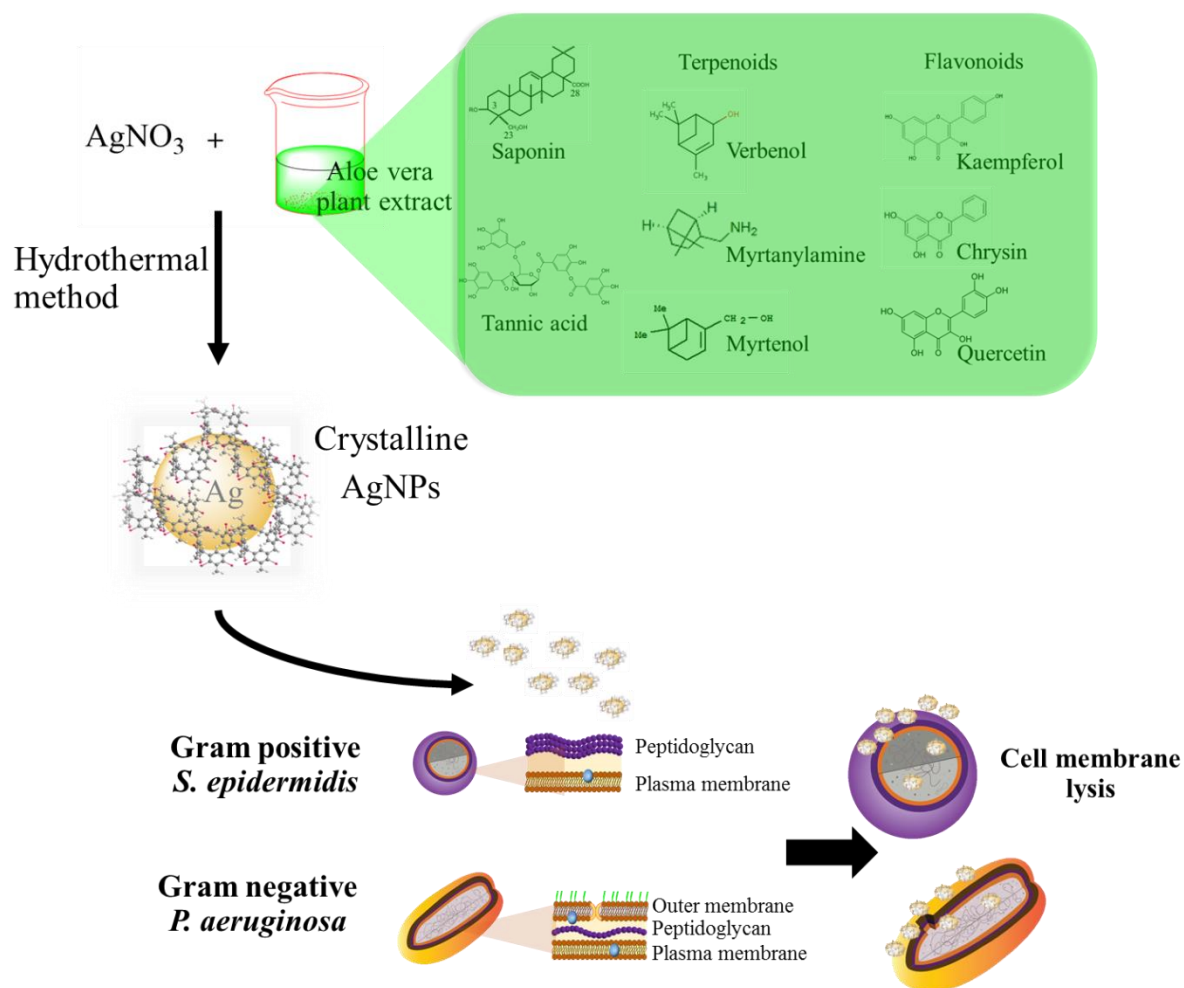
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480   **Figure 5 Illustration of proposed bacterial inactivation mechanism that may involve**

481   **nanocrystalline AgNPs@AV to disrupt the bacterial membrane.** In the hydrothermal

482   method, various organic compounds such as saponin, tannin, terpenoids, and flavonoids in the

483   aloe vera plant extract can be combined with AgNO$_3$ synthesizing AgNPs@AV. These

484   nanocrystals may accumulate at the cell membrane increasing its permeability, which

485   eventually results in the death of *P. aeruginosa* and *S. epidermidis*.

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488   **LIST OF TABLE CAPTIONS**

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

490   **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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492   *$p < 0.01$ compared with an $AgNO_3$ control

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