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September 17th, 2016

Dear Editors

We thank the reviewers for their generous comments on the manuscript "Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity"

Those comments of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. The following pages are our point-by-point responses to each of the comments of the reviewers as well as your own comments in the second revision.

Please find attached a revised version of our manuscript, which we believe that the manuscript is now suitable for publication in PeerJ.

We shall look forward to hearing from you at your earliest convenience.

Yours sincerely,

Dr. Patcharaporn Tippayawat
Lecturer of Clinical Microbiology

Dr. Apiwat Chompoosor
Assistant Professor of Chemistry

On behalf of all authors

***Reviewer 1 (Anonymous)***

***Basic reporting***

*The manuscript deals with a trendy topic, because of the worldwide interest in silver nanoparticles (AgNPs) and in natural bioactive substances. Unfortunately the AgNPs production process exhibits a broad range of toxicity in vertebrates and invertebrates, and 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 an interesting natural medicinal agent with multiple properties, including an antibacterial effect.*

***Experimental design***

*The used methods are correct and effective and the obtained results demonstrate an antibacterial activity on S. epidermidis and P. aeruginosa. The results showed that AgNPs had a high antibacterial effect, which depended on their synthesis conditions, particularly when processed at 100 oC for 6 h and 200 oC for 12 h. The cytotoxicity of AgNPs was determined using human PBMCs revealing no obvious cytotoxicity.*

***Validity of the findings***

*The work provides interesting and innovative findings, with applicative potential.*

***Comments for the author***

*The Authors have considered the given suggestions, mentioning at least the matter concerning the choice of testing only a limited number of microbial species.*

Thank you very much for these suggestions.

***Reviewer (Pasquale Massimiliano Falcone)***

***Basic reporting***

*Authors provided point-by-point responses to each of the comments with some minor weakeness.*

***Experimental design***

*No comments*

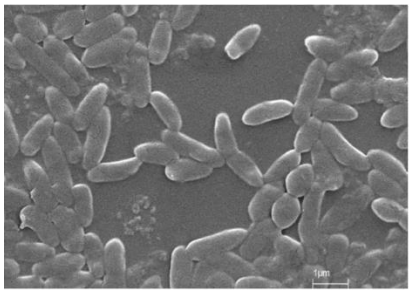
***Validity of the findings***

*Please make clear again the way you provide evidence of the inhibitory (reversible) and microbicidal (irreversible) effects of nanoclusters against the two investigated strains. A recovery procedure and subsequent incubation test might provide evidence of reversibility/irreversibility of nanocluster aggregation observed at 0.01mg/mL and 0.0025mg/mL for S. epidermidis and P. aeruginosa strains. Otherwise, you will provide evidence by microscopy of cell membrane damage (pores) for the S. epidermidis at 0.01 mg/mL or for that of the P. aeruginosa one at 0.0025mg/mL.*

Thank you very much for your suggestion. It is very important issue. We therefore perform the antibacterial activity of two high effective nanoparticles including 100$^{o}$C for 6h and 200$^{o}$C for 12h of AgNPs@AV at the concentrations of 0.01 mg/mL and 0.0025 mg/mL, respectively. The electron micrographs show the evidence of the particles damaging bacterial membrane as shown in SEM micrographs in the figure below. These results suggest that irreversible effects of the nanoclusters in the each different concentrations against the two pathogens.

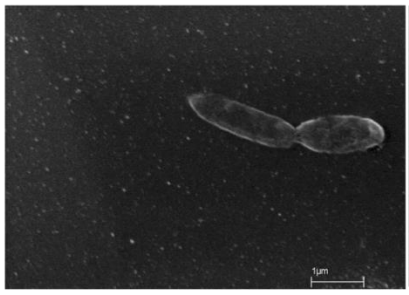
**Untreated microbial control**

***P. aeruginosa***

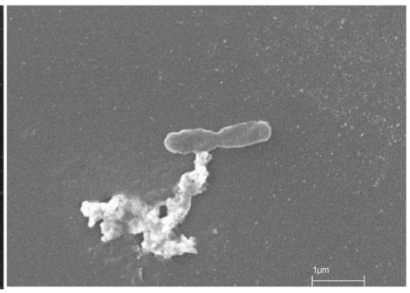


**AgNPs@AV 100°C for 6h**

**0.04 mg/mL**

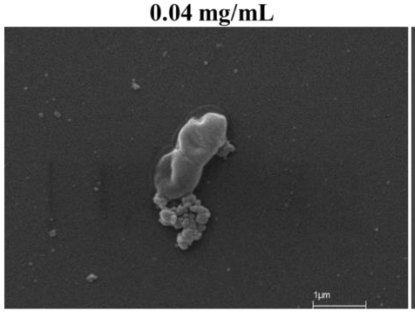


**0.0025 mg/mL**

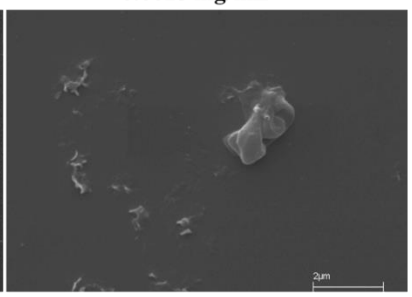


**AgNPs@AV 200°C for 12h**

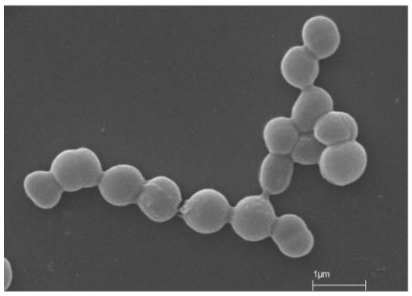
**0.04 mg/mL**



**0.0025 mg/mL**

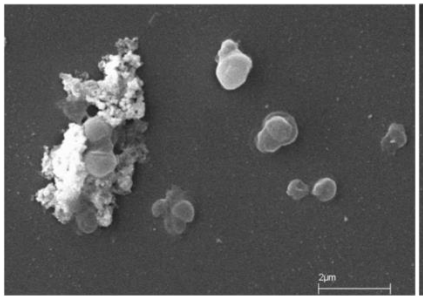


***S. epidermidis***

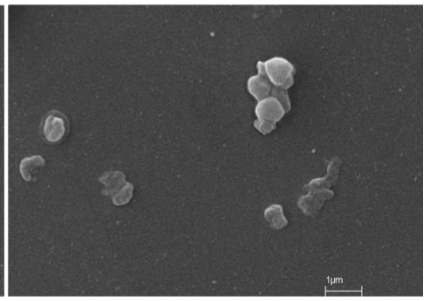


**AgNPs@AV 100°C for 6h**

**0.04 mg/mL**

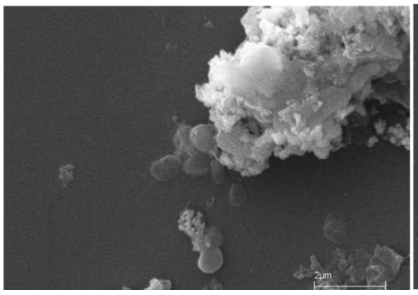


**0.01 mg/mL**

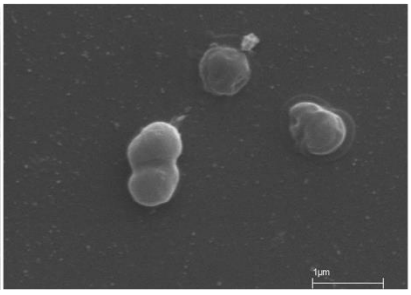


**AgNPs@AV 200°C for 12h**

**0.04 mg/mL**



**0.01 mg/mL**



***Comments for the author***

*Please make the following minor changes.*

*At line 109 - please change "vera" with "vera"*

*At line 202 - please change "stain" with "strains"*

Thank you very much for the prove reading. The words have been already corrected.

Changing the word "verla" with "vera" at line 99.

Changing the word "stains" with "strains" at line 202.