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July 1st, 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. In the following pages are our point-by-point responses to each of the comments of the reviewers as well as your own comments.

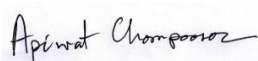
Please find attached a revised version of our manuscript, which we believe that the manuscript is now suitable for publication in PeerJ.

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

Yours sincerely,



Dr. Patcharaporn Tippiyawat
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 °C for 6 h and 200 °C 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

In general I think that a larger number of microbial species should have to be tested, so I think the Authors have to explain why they tested only two microbial strains and why they tested those two strains. The Authors have to consider and underline this aspect when they discuss the obtained results.

Agree with the point that you are concerned. Therefore, we have added in discussion with the point of the importance of the two stains.

In page 9 line 214-217, “These two stains are the opportunistic bacteria causing of nosocomial infection, moreover there are the virulent factors involving with antibiotic resistance (*Otto 2009, Livermore 2002*). Thus, our new product might be a material of choice to apply in antimicrobial activity instead of antibiotics”

Reviewer 2 (Anonymous)

Basic reporting

No Comments

Experimental design

No Comments

Validity of the findings

No Comments

Comments for the Author

Patcharaporn Tippayawat et al. report Green synthesis of silver nanoparticles in aloe vera plant extract prepared by a hydrothermal method and their synergistic antibacterial activity. The results are impressive especially for the antibacterial activity section. The article is suitable for this journal according to the content aspect. I recommend this article for publication. Nevertheless, I have some suggestions which are listed below.

- 1. It is better to provide some representative TEM images of the obtained Ag nanoparticles because the AgNP in the SEM images in this study were aggregated from which the diameter measurement seems difficult.*
- 2. If it is possible please provide the UV absorption spectra of the obtained colloid because the surface plasmon resonance band (PRB) of the Ag colloid can indicate some structural information.*

Thank you very much for this suggestion. Therefore we have already added these information in content and figure 2 in page 19.

1. The TEM image was added to the revised manuscript in page 8 line 191-193. The result was consistent with SEM images.
2. The absorption spectra were added to the revised manuscript in page 8 line 193-195 indicating that the maximum absorption was found at 420 nm and was attributed to the surface plasmon resonance of AgNPs.

Reviewer 3 (Pasquale Massimiliano Falcone)

Basic reporting

In the following some criticisms and weakness are summarized.

Some works from literature have been carried out focused on green biosynthesis of silver nanoclusters with antimicrobial functionalities, some of them are also cited into the manuscript. Authors must indicate with clarity what the gap of knowledge their results have been covering beyond the state of the art.

Dose-relationships between the concentration of the silver nanoclusters (as obtained at 100°C and 6h as well as at 200°C and 12h) and load of vital microbial cell are strongly recommended to be reported and discussed in depth in the manuscript.

In this regard, the bactericidal doses of the silver nanoparticlers were presented by MBC at 0.01 mg/mL for AgNPs fabricated at 100°C for 6h and 200°C for 12h against 10^8 CFU/ml *S. epidermidis*. The corresponding concentration was 0.0025 mg/mL for AgNPs fabricated at 100°C for 6h and 0.00125 mg/mL for those formed at 200°C for 12h against 10^8 CFU/ml *P. aeruginosa*. For this point was shown in the result part of antibacterial effects (page 9-10 line 228-237).

Also it was discussed in the part of discussion (page 12 line 294-303) that the AgNPs formed at 100°C for 6h and 200°C for 12h were found to be toxic to both gram-positive and gram-negative bacteria. It might due to the smallest size of 100°C for 6h AgNPs fabricated under these conditions which results a higher surface area to release the silver ion and aloe vera on bacteria. Whereas, the samples processed at 200°C for 12h had the largest size containing more amount of organic compounds, therefor it provided obviously effective growth inhibition of *P. aeruginosa* providing at lower two times of AgNPs fabricated at 200°C for 12h when compared with AgNPs fabricated at 100°C for 6h.

For a more easy reading, the manuscript should report the state of the art in the introduction section only.

Following this suggestion, the state of the art was emphasized that “this work has shown the use of naturally occurring compounds to be a reducing and stabilizing agent. This method is considered green synthesis. The resulting silver nanoparticles showed a synergism of aloe vera and silver nanoparticles on bactericidal effect. This hybrid nanomaterial provides an alternative material for using in antibacterials.” and addressed in page 4-5 line 101-104.

Experimental design

Authors should report more information about the experimental method used to estimate MIC levels as well as on how they accounted for the medium turbidity due to the presence of the silver nanoclusters during the growth/inhibition tests.

Thank you very much to give us the notice in this point. In the experiment, MIC is the lowest concentration of drug that inhibits the growth of the organism that it was observed the turbid or clear solution after the incubation time and also compared with broth alone control. Our results could provide the clear solution in the dose of MIC due to the killing bacteria aggregated with the nanoparticles underneath the broth solution. Furthermore, the sampling supernatants of each condition were measured with spectrophotometer.

Validity of the findings

A storage stability study about the antimicrobial ability for the two types of the more effective silver nanoclusters is lacking. No data are provided about nano structures and residual antimicrobial ability during storage intended for biotechnological or medical applications.

Agree with the important point. We have tested the reproducible activity of the nanoparticles by using well diffusion method that it was measured on diameter of the inhibition zone (mm) revealing the same inhibition zone for two months after the nanoparticles synthesis which it has already addressed in page 10 line 237-238.