Review of: "De-novo fabrication of sunlight irradiated silver nanoparticles and their efficacy against E. coli and S. epidermidis"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

In this study, SL-SpNPs were synthesized using *Salvadora persica* root extract (SPE) as reducing agent via sunlight (SL) irradiation method. The resulting SL-SpNPs were characterized using multiple techniques and their antibacterial efficacy was evaluated. The main contribution of this study is: it provided a cost effective and eco-friendly solar irradiation-based fabrication method, and the fabricated SL-SpNPs showed good antibacterial activity against *Escherichia coli* and *Staphylococcus epidermidis.*
There are a number of concerns with this study which include the following:

1. **Introduction:**
2. The last sentence of the first paragraph: “Therefore, in vivo studies are critical to determine the application of NPs and the synthesis approach will likely define their utility and efficacy in such applications”. The authors should explain (or cite previous references to illustrate) how synthesis approaches affect the efficacy of NPs in the applications, which will support the necessary of this study.
3. The fabrication of silver nanoparticles using *Salvadora Persica* (*S. Persica*) extract has been reported in many literatures (14, 17-20), and the sunlight irradiation method has also been reported (23-25). Therefore, this study is not innovative enough. Furthermore, the authors claimed that the antimicrobial assay on two bacterial pathogens (*E. coli & S. epidermidis*) were lacking in many of the previous studies, this is not true, especially for the *E. coli*, which is the most commonly used bacterium in antimicrobial assay of silver nanoparticles.
4. The last sentence of the Introduction: “Moreover, the methodology may provide new horizons in the disposal of NPs for applications where leaching of NPs is a challenge to address, for example, immobilized AgNPs in the polymers employed for water treatment”. The meaning of this sentence is unclear, confusing and obtrusive.
5. Table 1 is not *self-explanatory, and some presentations are errors.* For example:

The second column, 01, 03, etc., are these correct ?
The full names of the abbreviations ZOI and NA should be marked in footnotes.
The information in the eighth column “NPs for ZOI (μg/ml, ml etc.)” is confusing. The units of NPs are inconsistent among different studies and thus they are not comparable. Furthermore, the results of this

study are present in Table 1, which logically should not be listed in the Introduction.

1. **Methods**
2. In terms of methods, the research depth of this paper is not enough. This study did not analyze the chemical properties or composition (sugars, proteins, etc.) of the root extract, nor did the fabrication method specify the intensity of sunlight irradiation, all these conditions would affect the repeatability of the preparation.
3. In the process of preparing AgNPs by using biological materials to reduce Ag+ (silver nitrate, SN), centrifugation or dialysis are often used to remove the Ag+ that have not been reduced completely after the reaction, especially in the case of high concentration of silver nitrate (10mM) and short reaction time (10min). How can the author ensure the complete reduction of silver nitrate? If there is residual Ag+ in the system, the results of subsequent antibacterial experiments will be inaccurate.
4. The determination methods of MIC and MBC are not clearly stated, what are the specific concentration of gradient dilution ? In addition, the determination method of MBC is not correct. MBC is the minimal antibacterial density necessary to kill bacteria, that is, bactericidal as opposed to merely bacteriostatic densities. The next higher NPs concentration doesn't necessarily kill bacteria.

1. **Results**
2. As shown in Figure 4, the ZOI values of SL-SpNPs with different concentrations were similar, and no significant difference among the ZOI values, which was a suspicious result. If this result is true, how can the MIC value of SL-SPNPS be measured? What are the relationships between these concentrations of SL-SpNPs in Figure 4 and their MIC or MBC values ? The concentration units of SL-SpNPs in the whole paper should be unified for comparison.
3. As shown in Figure 5, SL-SpNPs showed a similar antibacterial effect, which is inconsistent with previous conclusions. It is generally believed that the antibacterial ability of silver nanoparticles is much higher than that of Ag+.

1. **Discussion**
2. Line 15, Figure 1b should be Figure 1a.
3. The third paragraph of Discussion, Line 8, "Further, MIC and MBC values are considerably lower (2 orders of magnitude) as compared to Shaik et al.[19] demonstrating higher antimicrobial potential and potential low toxicity. However, these values are greater than previous study using heat based synthesis of AgNPs[20]". The analysis here is wrong. Lower MIC and MBC values indicate stronger antibacterial performance, not vice versa.